LANE MEDICAL LIBRARY STANFORD
D581 .C88 1928 2 OLD
Special cytology : the form and function

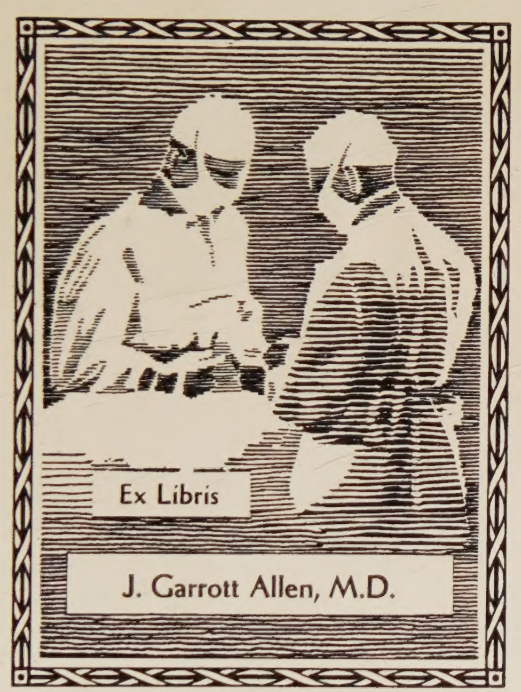
24503091461

MAR 2 3 1967

LANE
MEDICAL
SEAL OF THE LELAND STANFORD JUNIOR UNIVERSITY
SEMPER VIRENS
1885
LIBRARY

Gift

LANE MEDICAL LIBRARY
300 PASTEUR DRIVE
PALO ALTO, CALIF. 94304
D 581
C 88
1928
Vol 2



SPECIAL CYTOLOGY

VOLUME TWO

SPECIAL CYTOLOGY

THE FORM AND FUNCTIONS OF THE CELL IN HEALTH AND DISEASE

A Textbook for Students of Biology and Medicine

Contributors

LESLIE B. AREY
PERCIVAL BAILEY
R. R. BENSLEY
C. H. BUNTING
ALEXIS CARREL
A. E. COHN
G. W. CORNER
E. V. COWDRY
HAL DOWNEY
G. CARL HUBER
J. ALBERT KEY
E. B. KRUMBHAAR
ALBERT KUNTZ
LEO LOEB
C. C. MACKLIN
M. T. MACKLIN
E. F. MALONE
F. C. MANN
DAVID MARINE
A. A. MAXIMOW
E. B. MEIGS
C. W. METZ
W. S. MILLER
EUGENE L. OPIE
WILDER G. PENFIELD
A. T. RASMUSSEN
J. PARSONS SCHAEFFER
G. E. SHAMBAUGH
P. G. SHIPLEY
G. N. STEWART
D. R. STOCKARD
D. L. STORMONT
FREDERICK TILNEY
T. WINGATE TODD
G. B. WISLOCKI

Edited by

EDMUND V. COWDRY
The Rockefeller Institute for Medical Research

693 ILLUSTRATIONS

VOLUME TWO

PAUL B · HOEBER · INC
NEW YORK · MCMXXVIII

LANE LIBRARY. STANFORD UNIVERSITY

Copyright, 1928
By PAUL B. HOEBER, Inc.
All Rights Reserved

Published March, 1928

Printed in the United States of America

88
928
1.2

TABLE OF CONTENTS

PAGE

PREFACE vii

SECTION

I. INTRODUCTION . . . . . *Alexis Carrel* 1

II. THE SKIN AND ITS DERIVATIVES . . . . . *E. V. Cowdry* 11

III. THE MUCOUS MEMBRANE OF THE NASAL CAVITY AND THE PARANASAL SINUSES . . . . . *J. Parsons Schaeffer* 45

IV. THE EPITHELIUM OF THE LOWER RESPIRATORY TRACT *William Snow Miller* 69

V. THE SALIVARY GLANDS . . . . . *D. L. Stormont* 89

VI. THE GASTRIC GLANDS . . . . . *R. R. Bensley* 137

VII. THE INTESTINAL EPITHELIUM *Charles Clifford Macklin and Madge Thurlow Macklin* 169

VIII. THE CYTOLOGY OF THE LIVER AND ITS FUNCTIONAL SIGNIFICANCE *Frank C. Mann* 203

IX. CYTOLOGY OF THE PANCREAS . . . . . *Eugene L. Opie* 239

X. THE ERTHYROCYTE . . . . . *E. B. Krumbhaar* 273

XI. THE LYMPHOCYTES AND PLASMA CELLS . . . *Alexander A. Maximow* 319

XII. THE MYELOBLAST . . . . . *Hal Downey* 369

XIII. THE GRANULAR LEUCOCYTES . . . . . *C. H. Bunting* 401

XIV. THE MACROPHAGES OR HISTIOCYTES . . . . *Alexander A. Maximow* 425

XV. THE STRUCTURE OF THE HYPOPHYSIS CEREBRI OF MAN AND OF THE COMMON LABORATORY MAMMALS . . . . . *Percival Bailey* 485

XVI. THE PINEAL GLAND . . . . . *Frederick Tilney* 501

XVII. THE THYROID, PARATHYROIDS AND THYMUS . . . . . *David Marine* 549

XVIII. THE SUPRARENAL BODIES . . . . . *G. N. Stewart* 621

XIX. RENAL TUBULES . . . . . *G. Carl Huber* 661

XX. CARTILAGE AND BONE . . . . . *P. G. Shipley* 703

XXI. THE SYNOVIAL MEMBRANE OF JOINTS AND BURSAE . . . *J. Albert Key* 735

XXII. STRIATED AND SMOOTH MUSCLE . . . . . *Edward B. Meigs* 767

XXIII. CARDIAC MUSCLE . . . . . *A. E. Cohn* 805

XXIV. THE SPECIALIZED SYSTEMS OF THE HEART . . . . *T. Wingate Todd* 851

XXV. VISUAL CELLS AND RETINAL PIGMENT . . . . *Leslie Brainerd Arey* 887

XXVI. CYTOLOGY OF THE INTERNAL EAR . . . . . *George E. Shambaugh* 927

XXVII. THE INTERNAL ARCHITECTURE OF NERVE CELLS . . . *E. V. Cowdry* 963

XXVIII. THE GENERAL RELATION OF HISTOLOGICAL CHARACTER TO FUNCTION IN MAMMALIAN NEURONS . . . . . *E. F. Malone* 989

XXIX. THE SYMPATHETIC NERVE CELLS . . . . . *Albert Kuntz* 1007

SECTION PAGE

XXX. Neuroglia and Microglia. The Interstitial Tissue of the Central Nervous System . . . . . *Wilder Penfield* 1031

XXXI. The Cytology of the Cerebrospinal Pathway . . *G. B. Wislocki* 1069

XXXII. Cytology of the Ovum, Ovary and Fallopian Tube. *G. W. Corner* 1109

XXXIII. Cellular Changes in the Fluid of the Mammalian Vagina *Charles R. Stockard* 1151

XXXIV. The Cytology of the Mammary Gland. . . . . *Leo Loeb* 1173

XXXV. Interstitial Cells of the Testis. . . . . *A. T. Rasmussen* 1209

XXXVI. The Male Germ Cells. . . . . *Charles W. Metz* 1257

XXXVII. The Seminal Vesicles, Prostate and Bulbo-urethral Glands *Charles Clifford Macklin* 1301

Index 1327

Section XX

CARTILAGE AND BONE

CONTENTS

Section XX

PAGE

I. Cartilage 705
1. Hyaline cartilage 705
2. Perichondrium 705
3. Cartilage matrix. 706
4. Cartilage cell 706
5. Function of cartilage cell. 707
6. Circulation in cartilage. 708
7. Supply of nerves to cartilage 709
8. Further story of cartilage cell. 709
9. Yellow elastic cartilage. 711
10. White fibrous cartilage. 711
II. Bone 712
I. Cytology of Undecalcified Bone. 715
1. Haversian systems. 715
2. Lamellae. 715
3. Lacunae and canaliculi. 718
4. Structure of lamellae. 718
II. Histology of Decalcified Bone. 719
1. Cellular structure 720
2. Lamellar structure. 722
3. Haversian system 722
4. Variations in cytology of bone. 723
5. Phenomena which accompany resorption of bone 724
6. Development of bone in embryo. 727
7. Development of "membrane bones" 731
8. Calcification 731
9. Halisteresis. 732
III. Bibliography 733

Section XX

CARTILAGE AND BONE

P. G. SHIPLEY

I. CARTILAGE

CARTILAGE, one of the supporting tissues of the body, is of mesodermal origin. It is made up of a ground substance or *matrix* and the *cartilage* cells or *corpuscles*. The condition of the matrix determines the division of the tissue group into three varieties, viz. hyaline, white fibro, and elastic cartilage, according as the ground substance is free from fibrous structures, or contains collagenous, or elastic fibers.

1. *Hyaline cartilage:*

By far the greatest amount of the cartilage of the body is hyaline cartilage. It is found in the trachea, the bronchi, and the costal cartilages. It covers joint surfaces and may be regarded either as a preosseous tissue or as a substitute for bone, fulfilling the functions of the latter tissue. The amount of this substance in relation to the mass of body tissues varies with the age of the individual. In the fetus the entire skeleton is originally represented by cartilage (*fetal cartilage*) but as life progresses cartilage is steadily replaced by bone. As growth proceeds the diaphyses or shafts of the units of the cartilaginous skeleton become ossified and cartilage remains only in the epiphyses. The epiphyseal cartilages become epiphyseal discs of cartilage, and are progressively narrowed as ossification proceeds until they are at length destroyed in the course of the growth of the bones and the epiphyses become united to the shafts.

2. *The perichondrium:*

Hyaline cartilage is always surrounded, except as surfaces within joints are concerned, by a perichondrium which in the case of epiphyses and periarticular cartilage is directly continuous with the periosteum. This membrane (Fig. 297) is in the main of white fibrous tissue but contains a large number of yellow elastic fibers. The cells, which must be regarded as precartilage, are for the most part flattened elements and indistinguishable from the ordinary fibroblast. These cells have round or oval nuclei and abundant cytoplasm and contain, beside mitochondria of varying size and shape, small droplets of fat and small vacuoles and granules of segregation. In the inner layers close to the cartilage the cell bodies become very numerous as the result of mitotic division, so that a *chondrogenetic* layer of the perichondrium is formed. The cells in this layer, because of the rapid divi-

sion, are of smaller size than the parent connective tissue cells and are converted directly by metamorphosis into the cartilage cell itself. The perichondrium is vascularized by a plexus of small blood vessels, especially important in the embryo since it gives origin to the blood vessels which run in the so-called cartilage canals.

3. *The cartilage matrix (hyaline):*

The cartilage itself consists of a matrix which is extremely elastic and very dense and firm. Chemically it is in a large part composed of the special protein, gelatin, but it contains a mucin also under ordinary conditions.

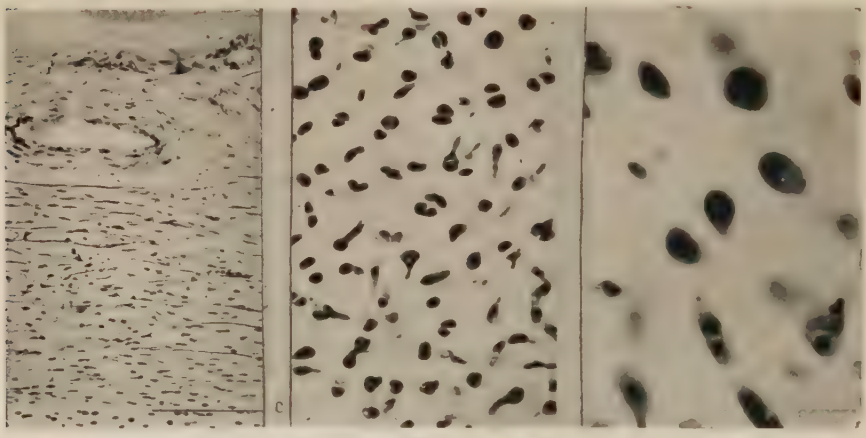


FIG. 297. -Perichondrium: c, osteogenetic layer.

FIG. 298.—Cartilage cells. × 150.

FIG. 299.—Cartilage cells. × 500.

On examination with the microscope it appears to be homogeneous and quite without structure. However, it has been stated that the hyaline matrix may be split up by appropriate treatment into fibers which run at right angles to the articular surface in the articular cartilages of long bones, and parallel with the long axis of the rib in the costal cartilages. The writer has never seen or been able to demonstrate either these fibers or the canaliculi which are said by some authors to traverse the matrix in all directions. The latter have been assumed to account for the penetration of cartilage by tissue fluid holding in solution food substances and metabolites. It seems, however, much more probable that this fluid migration is the result of physico-chemical phenomena and that diffusion plays a large part in the process.

4. *The cartilage cell:*

The cartilage cell is characteristic both as regards its form and the arrangement of these elements in the tissue. These cells (Figs. 298, 299) may

be round, oval or bluntly angular, but in normal resting cartilage the most characteristic cell is one which is bean shaped, viz. flattened on one side and irregularly oval in the remainder of its contour. The cytoplasm in the adult cell is clear or has a ground glass appearance. It contains a great deal of fat usually in the form of fine droplets but these may coalesce and increase in size until the cartilage corpuscle resembles exactly a cell in adipose tissue. Preagonal staining with cresyl violet or neutral red stains only small segregation bodies. The mitochondria are for the most part present as long wavy threads though short rods and granules of mitochondrial substance may occur. In life the cartilage cell entirely fills the cavity in which it lies in the matrix but the process of fixation frequently results in its shrinkage and the separation of the cytoplasm from the surrounding hyaline substance. The nucleus of the cell is vesicular and in the fresh state is much more homogeneous than the cytoplasm. When fixed and stained it may be seen that the chromatin is very abundant. There are usually two nucleoli but frequently only one is found.

Just beneath the perichondrium the cartilage corpuscles lie close together and they are smaller than the cells found toward the center of the mass. All transition forms may be found between the chondrogenetic cell and the true cartilage corpuscle at the under surface of the perichondrium. However, the transition takes place very rapidly so that the area of immature cartilage beneath the perichondrium is very thin. There is a sharp functional separation at the epiphyseo-diaphysial line between perichondrium and periosteum so that the same membrane may be seen on either side of the line, giving rise in one case to osteoblasts and in the other to cartilage cells.

5. The function of the cartilage cell:

The matrix is the product of the activity of the cartilage corpuscle. The cell builds cartilage about itself just as the osteoblast lays down osteoid tissue and buries itself in an investure of its product. At first there is only one corpuscle in each lacuna of matrix but the new cells divide rapidly so that the characteristic resting cartilage has two cells in a lacuna with their flattened surfaces in apposition. These paired cells may redivide in such manner that the lacuna may contain three, four, or more. Each of the cells in a lacuna then begins to produce matrix so that in a short time the group may be divided by partitions of this material which increase in thickness and again lacunae are formed containing each but a single cell.

Apparently there is an intermediate stage in the formation of the matrix since it is possible to see a "capsule" between the cytoplasm of some of the cartilage cells and the periphery of the lacuna in which they lie. The mechanism of the formation of the matrix has never been satisfactorily settled but there is much to be said for the view that the ectoplasm of the cell is used in its manufacture. However if this be the case the growth of the

cytoplasm must be permanent since there is an indefinite increase possible in the amount of matrix. There is a remarkable analogy between the growth of bone and of cartilage which is only in abeyance under certain pathological conditions. In severe cases of syphilitic osteochondritis for example ossification and absorption of bone are completely inhibited but growth of the cartilage goes on uninterruptedly. It may be said in general, nevertheless, that there is a remarkable correlation between the amount of osteoid in a bone and the amount of matrix in the cartilaginous epiphysis. In those pathological conditions such as rickets in which there is a great overproduction of osteoid tissue and the bone corpuscles are small and few in number, the matrix of the cartilage is redundant and there is a corresponding diminution in the size of the cartilage cell. On the other hand when bone or osteoid is very cellular the matrix of the epiphyseal cartilage is produced in small amounts and the lacunae are crowded close together.

6. *The circulation in cartilage:*

Cartilage in the adult is avascular. Large masses such as the costal cartilages may be invaded by an occasional blood vessel, apparently because the mass of tissue is so great that an adequate supply of food substances by diffusion from the marrow of the adjacent bone is impossible. This does not hold good for the same tissues in the fetus. When the cartilaginous skeleton is first laid down it is as in the adult avascular. Soon, as growth goes on and after osteogenesis is well under way, blood vessels invade the cartilage just as they do the primitive bone carrying with them a covering of perichondrium. Growth of these vascular canals is at a right angle to the long axis of the epiphysis for a varying distance, but the direction soon changes and the vessels turn to grow toward the line of ossification of the bone to which the epiphysis belongs. At first the vessels form an indifferent network of capillaries, a simple outpouching of the perichondrial capillary plexus, but soon there develops differentiation into artery and vein connected at the growing end by a knot of capillaries which bears a striking resemblance to the renal glomerulus. While there is no certain arrangement of these structures which are known as cartilage vessels they tend to enter the epiphyses of different bones in a more or less definite pattern. Their arrangement however is certainly not "etagen formig" as has been stated by some of the German writers who have studied them.

Their course is continued until they are met by the provisional zone of calcification through which they descend or ascend as the case may be to anastomose with the capillary terminations of the vascular tree of the bone marrow. These cartilage vessels canalize the matrix at relatively wide distances apart. They are enclosed in tunnels, surrounded by delicate connective tissue, in which numerous leucocytes are formed, which is known as the cartilage marrow. A section across one of these "cartilage

canals" demonstrates according to its level, artery and vein or capillary network, one or more lymphatic vessels and a fine nerve fiber or two. At not infrequent intervals along the course of the canal the artery and vein communicate directly by means of single straight capillary vessels. A more efficient method of slowing the flow of blood is difficult to imagine and diffusion of substances in both directions through the walls of these vessels must be greatly facilitated thereby. It may be well to say in this connection that the cartilage on the borders of these canals is different in its staining reaction from that of the rest of the tissue. It is colored less intensely by basophilic dyes. Moreover it is not so rich in matrix and the characteristic arrangement of the cells is disturbed. The cells themselves tend to assume a spindle or flattened form resembling that of the fibroblast.

Equal in importance to the nutritive function which the above described structures must undoubtedly subserve is the rôle which they play in the development of the centers of ossification in the epiphyses. The entrance of vessels from these canals into a centrally placed area of calcified cartilage is the beginning of ossification of the epiphyseal center, in the development of which these vessels represent those which in process of primitive osteogenesis bud from the sub-periosteal plexus to furnish the vascular tree in the new bone (see below).

7. *The supply of nerves to cartilage:*

Cartilage is notoriously insensitive and except for those fibers described above as contained in the cartilage canals is without a nerve supply.

8. *The further story of the cartilage cell:*

If a section through the cartilaginous epiphysis and the epiphyseal end of a growing bone is examined under the microscope the cartilage cell may be seen to undergo a remarkable series of changes as the cartilage is encroached upon by the advancing osseous tissue. The cells first become flattened with their surfaces parallel to the epiphyseo-diaphyseal line (Fig. 300). Their characteristic arrangement is lost and they are grouped into columns of single cells and masses of these columns are formed. The character of the matrix changes. Between the individual cells it becomes condensed either by loss of water or actual resorption, and more intensely basophilic in its tinctorial reactions (Fig. 300). Many of the changes which have been described as occurring in the individual cells during this metamorphosis —the formation of the proliferative zone of cartilage— are very doubtful since because of the mechanical difficulties in the way of the penetration of fixing reagents examination of the cells themselves in anything like their natural condition is to all intents and purposes impossible.

In specimens of growing cartilage and bone prepared in the ordinary way the cells in the so-called preparatory or proliferative zone appear after a

preliminary stage of swelling or expansion to shrink and undergo granular degeneration. The nuclei seem pyknotic, the cytoplasm more and more intensely basophilic until in the area which is undergoing calcification the cartilage cell is represented by a ragged, intensely basophilic shrunken mass lying free in the center of its *primary areola*, all traces of cell structure having become indistinguishable. If these appearances are accepted literally they can only be interpreted to mean the death of the cartilage cell. I believe that much of this picture is artifact, the result of necessarily

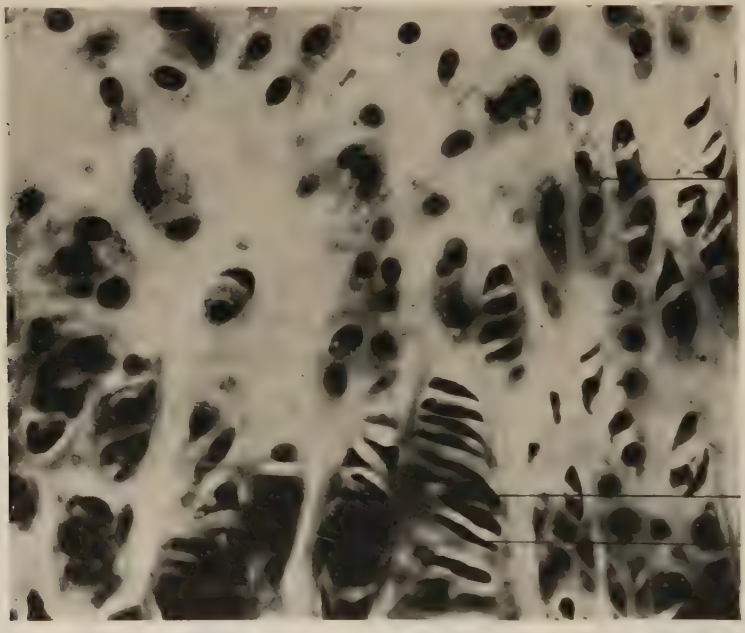


FIG. 300.—Proliferative zone of cartilage: p, proliferating cartilage cells; b, basophil matrix; c, flattened cartilage corpuscle.

poor fixation of the cells. Calcified cartilage offers great obstacles to the passage of any fixing fluid and the slow penetration of the latter would allow time for degenerative changes before precipitation of the proteins brings them to a halt. It appears likely from study of cartilages from which calcification is absent that instead of shrinking into a compact pyknotic mass the cartilage cell in the area of provisional calcification actually becomes larger and that its nucleus becomes more vesicular and poorer in chromatin and the cytoplasm stains slightly more deeply than normally.

The next step in the process is calcification of the matrix to form the so-called provisional zone of calcification (Fig. 301). Lime salts, principally the tertiary calcium phosphate $(Ca_3PO_4)_2$ are precipitated in the inter-

cellular matrix between the areolae. At first the precipitate is granular but in a short time the former matrix has become homogeneously and densely infiltrated with lime and is the most primitive form of calcareous tissue—calcified cartilage. The calcified material stains intensely with basophilic dyes except cresyl echt violett.

The biological significance of the cartilage corpuscles is far from clear in this whole process. Personally I am inclined to look upon the formation of the proliferative zone and calcification and the phenomena leading up to it as a resting stage in the life of the cartilage cell and perhaps analogous to encystment in a protozoan.

FIG. 301.– Zone of provisional calcification: pz, proliferative zone; a, primary areola with cartilage corpuscle; c, calcified matrix.

9. *Yellow elastic cartilage:*

This variety of cartilage is found in the pharynx, Eustachian tube, and the cartilages of the ear. It is only a modified form of hyaline cartilage from which it differs in that the matrix is filled with yellow elastic fibers which proceed in all directions. There is a clear space in which these fibers are not found about each of the cells. The arrangement of the latter is not so constant as in hyaline cartilage. The fibers are irregular in length, some of the material which takes the characteristic stains for elastic tissue consists of mere granules which are assumed to be immature fibers.

10. *White fibrous cartilage:*

This tissue represents a mixture of cartilage and white fibrous tissue, it is extremely tough, but is more elastic than is the ordinary hyaline variety of certilage. Histologicallyit consists of an hyaline matrix in which are

embedded wavy bundles of white fibers. The cells which are indistinguishable from those of hyaline cartilage lie in a space or capsule in the matrix the vicinity of which is free from fibers. The cells are often arranged in straight rows just as the cells in tendons are. White fibrous cartilage seldom occurs alone but is usually mixed with the hyaline variety. The tissue is found in interarticular discs of cartilage such as the semilunar cartilages of the knee, in cartilages in symphyses, lining the grooves in bone through which tendons pass, and in small sesamoid cartilages in the tendons themselves.

II. BONE

It has become customary in the past to consider bone as a tissue which is changeless and subject to little if any variation in its minute structure. Anatomists have realized in the past that the gross architecture of a bone might undergo a considerable degree of transformation to compensate for the changes in direction of the lines of force which resulted from the disturbance in the contour of the bone following the healing of fractures in malposition. They were familiar, moreover, with the atrophy which is the result of old age or of extreme and long continued disuse such as is found in individuals with limbs paralyzed from poliomyelitis or other causes.

Pathologists have been cognizant for years of the rapidity of the resorption of bone before the encroachment of rapidly growing masses of soft tissue, e.g. aneurysms and tumors. And as the anatomists recognized the atrophy of disuse and old age they were familiar with the hypertrophy of lues venerea and the rarefaction of bone which is the result of pyogenic osteomyelitis or osteitis fibrosa cystica. It is only of late, however, that there has been any recognition accorded the fact that the microscopic anatomy of the skeleton is subject to complete and rapid reorganization and that in small animals the period of reconstruction is a matter not of months or weeks but of days.

Perhaps there is no better illustration of the old saying "Pathologia physiologium illustrat" than the knowledge of the physiology of normal bone which has been derived in the last few years from studies made on bones of animals so fed as to induce in them a pathological metabolism of the different foodstuffs with which the tissues are normally supplied. These studies forced on the student of microscopic anatomy the realization that one, instead of being almost completely stable, is one of the most labile of all the tissues of the body and one in which every change of tissue metabolism, diet and exercise is recorded almost, perhaps even literally, from day to day. Of course there is unfortunately at the present time no known way of recording the response of bone to single stimuli of minor intensity but the result of their summation can leave no doubt that the bone cell

responds in its own way to changes in its environment as rapidly and decidedly as the muscle cell.

Perhaps the work of Mcewen (1923) on the growth of the antler did more than any other single study to call attention to the rapidity with which bone can be made by the osteoblast under completely physiological conditions.

Bone may exist in the body of an animal as an exoskeleton, for example the antler of the deer, as cutaneous bone in the skin of the armadillo and lastly and most commonly as an endoskeleton. It consists *in essentiam* of an organic matrix (osteoid) tissue in which the bone cells* which have formed the osteoid are embedded. Various inorganic salts, chiefly tertiary calcium phosphate, are deposited in the organic matrix but not in the cells themselves.

Since, as has long been known to pathologists and clinicians, any single bone may react independently of the rest of the skeleton to stimuli which affect it (e.g. disuse atrophy or trophic osteoporosis affecting a single limb) it is necessary to think of each bone as a separate organ in a great organ system.

In the main the functions of bone are (1) supporting and (2) protective. The first is exercised in relation to the entire body, the latter in the defense of specialized and delicate tissues as the central nervous system and the bone marrow.

The physiology of the bones *inter se* is chiefly concerned with phenomena of growth (osteogenesis) and its corollary resorption. The growth of bone begins early in the life of the fetus and continues until brought to a standstill by the death of the organism as a whole. Growth is brought about (1) by increase in the number of cells, (2) by increase in the amount of osteoid tissue and (3) by calcification of the latter—the last step in the process of osteogenesis. If the above mentioned processes of osteogenesis are considered in detail it will be seen that increase in the number of cells may come about through (1) proliferation of previously formed bone cells (osteoblasts) or (2) by the conversion of other cells to the bone forming type. Osteoid tissue is a product of cellular activity and an increased osteoid results from an increased osteoid forming capacity of individual bone cells or from a normal osteoidogenesis of a normal or increased number of osteoblasts.

Resorption of bone, which goes hand in hand with but in varying relation to osteogenesis, occurs through (1) the agency of the osteoblast (2) blood vessels and perhaps to a slight extent (3) certain large multinucleated cells called osteoclasts.

* These cells are known as osteoblasts during their existence as free cells before they formed bone or after they have completed the resorption of the bone in which they are an integral part. When imbedded in the bone or osteoid they are called bone cells or corpuscles. The differentiation is functional.

Bones may be either "long bones" or "flat bones." Examples of long bones are the femur, tibia, radius, ulna, etc. The scapula and the bones of the calvaria are flat bones. Moreover, bones may be membrane bones as for example the os frontalis or the squama temporalis, or cartilage bones as are the femur, ribs, tibia, et al. Now if a growing bone, let us say for example the tibia of a newborn baby, be examined it will be seen at once that it is divided into parts. There is a shaft in the center (the diaphysis) composed of bone and at either end an epiphysis or head of cartilage, divided each from the other by the epiphyseo-diaphyseal line. The bone is covered by a tough very adherent membrane (periosteum) carrying by blood vessels and nerves which enter the bone and continuing with a more delicate but equally adhesive membrane (perichondrium) which surrounds the cartilaginous epiphysis. The inner or visceral surface of the periosteum when it has been stripped from the bone, has a shaggy appearance due to projections of the membrane which pass through the cortex of the bone itself. The surface of the bone stripped of its periosteum appears dull and dry and can be seen if closely examined to be pierced by innumerable small holes from which the perforating processes of the periosteum and tiny blood vessels have been withdrawn. If the bone is now split longitudinally from end to end the same divisions into diaphysis and epiphyses are evident on the cut surface (Fig. 313). If the bone chosen is from a somewhat older child, let us say one during its second year of life, an island of bone will be seen in the center of the epiphyseal cartilage. This is the epiphyseal center of ossification. As the development of the bone progresses other centers of ossification appear in cartilage, for example that of the tubercle of the tibia and grow until they unite with the shaft of the bone. The epiphyseal center enlarges in all directions as the age of the child increases, and the bone of diaphysis grows toward it until diaphysis and epiphysis are separated only by a thin layer of cartilage—the epiphyseal disk of cartilage—which is eventually broken through and disappears. The epiphysis is then said to be united to the shaft.

Examining the shaft of the bone from without inward it will be seen that immediately beneath the periosteum there is a thick dense trumpet-shaped tube of bone (the cortex), the solid walls of which (*compacta*) are thickest at the middle of the shaft and are thinner as the tube flares out into a bell at the epiphyseo-diaphyseal junction. At either epiphysis this tube is quite filled with a spongy mass (*the spongiosa, cancellous bone*) of delicate spicules (*trabeculae*) of bone. These are straight, very slender and roughly parallel to the long axis of the shaft at the epiphyseo-diaphyseal junction but further down in the shaft they become more massive, exist in smaller numbers and tend to form a lattice work. The largest trabeculae of the spongiosa are arciform and their foundations spring from the wall of the cortex. As the center is approached the spaces between the trabeculae of the spongiosa become wider as the result of physiological resorption (*canalization*) and in the very center the trabeculae are almost or entirely wanting. The spaces between the trabeculae in the spongiosa and the cavity in the center of the bone are filled in newly born children with dark red jelly-like bone marrow (red marrow) or in older individuals with fat (yellow marrow) or a greyish mixture of marrow and fat.

The region of most rapid growth just shaftward of the epiphyseal cartilage where the trabeculae are most delicate and straightest has been called the metaphysis. This region has no distinct dividing line from the shaft in normal bones, but under pathological conditions such as rickets the metaphysis is a very important region in the bone and is easily made out on examination.

Bone may be prepared for study with the microscope in three ways. The organic matter, the marrow, cells, osteoid, etc., may be removed by drying, ashing or autolysis or bacterial or chemical digestion. The inorganic salts may be removed by soaking in acids or in citrate of magnesia, until the

bone becomes pliable and elastic. Or finally the undecalcified tissue after fixation may be impregnated with rosin and the organic and inorganic material may be preserved and studied in the same preparation.

I. CYTOLOGY OF UNDECALIFIED BONE

1. *Haversian systems:*

If a sawn section through the compacta of a bone is examined with a glass giving a magnification of × 10 the section will be seen to be perforated throughout its area with fine holes. With the aid of higher magnification these will be seen to be sections of short canals cut longitudinally, transversely and in every degree of obliquity. These are the *Haversian canals*

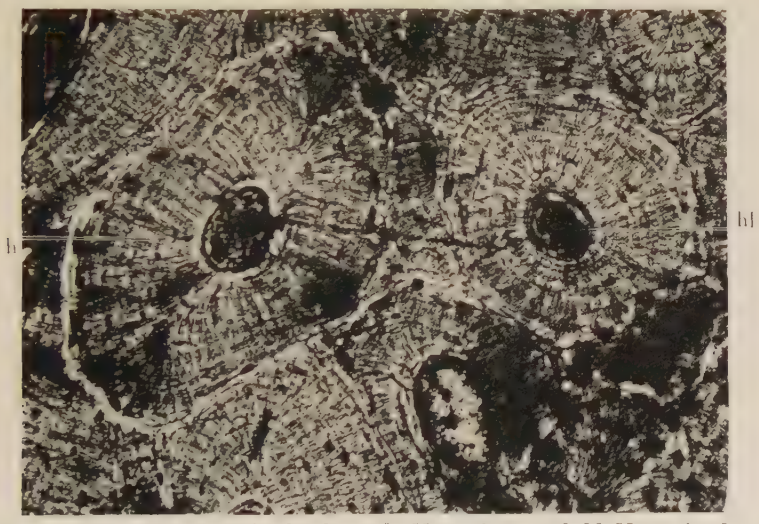


FIG. 302.—Haversian systems in dry bone: h, Haversian canal; hl, Haversian lamellae.

(Fig. 302) on which the architecture of the bone is based. They are short and intercommunicate freely by short branches. Those nearest the compact cortex are narrower than those nearest the medullary cavity. Those which are in the dense bone beneath the periosteum run parallel for the most part with the surface of the bone onto which surface they open by tiny pores. The inner ones open into the medullary cavity. These canals contain the vascular system of the bony tissue, which will be discussed in detail later.

2. *Lamellae:*

Between and about these Haversian canals the bone can be seen to be built in layers which are called *Lamellae* (Fig. 303). Just beneath the periosteum these strips run parallel with the outer surface and they are known as

circumferential or periosteal lamellae. Cross sections of the bone show the Haversian canals to be surrounded, each by a number of lamellae concentrically disposed. These lamellae are separated each from the others by narrow spaces of approximately equal width which widen at more or less regular intervals to form *lacunae*. The lamellae usually surround the Haversian canal completely but this is not uniformly the case since some may be seen to terminate without forming a complete circle. These Haversian lacunae with the canal about which they were built up form what is known as an Haversian system. According as a section is cut directly transversely

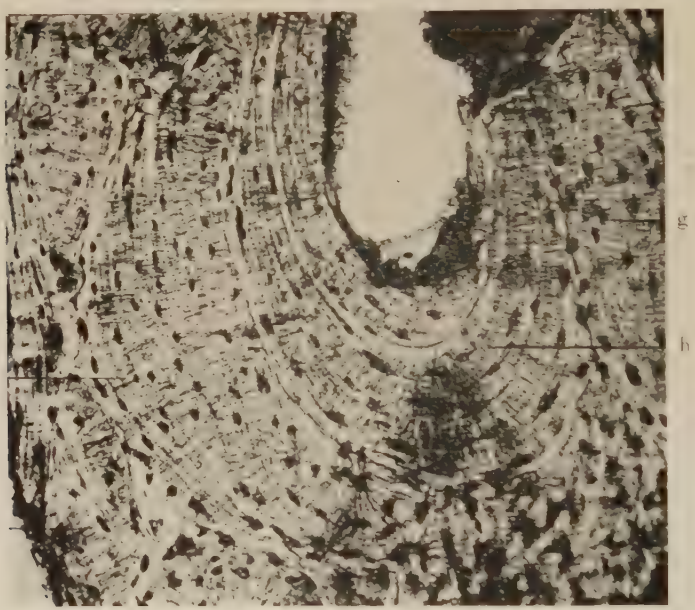


FIG. 303.—Section of dry bone showing, h, Haversian lamellae; g, ground lamellae; c, circumferential lamellae.

or with greater or less obliquity as regards the longitudinal axis of the bone the majority of the Haversian lamellae and the Haversian canals will appear circular or of more or less long oval form. In sections cut parallel with the long axis of the bone, however, the greater number of canals will be seen as tubes opened longitudinally and the longitudinal sections of the lamellae which are in reality hollow tubes will be found running parallel to the canal on either side of it. In whichever direction the section is cut there will be found of course Haversian systems cut in all possible ways.

Between the Haversian systems and filling the interstices between them lamellae will be seen which are irregularly disposed. These are called *ground lamellae*.

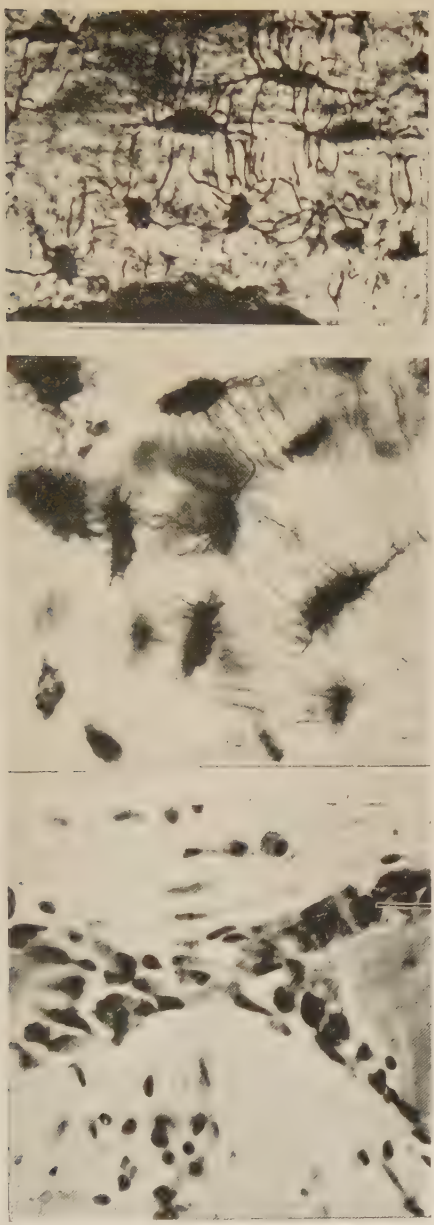


FIG. 304.—Lacunae and canaliculi in dry bone.
FIG. 305.—Bone corpuscles showing processes stained with gold chloride.
FIG. 306.—Columnar osteoblasts surrounding trabeculae: o, osteoblasts.

Haversian canals in small numbers which pass through the subperiosteal lamellae to open beneath the periosteum on the surface of the bone may be without surrounding lamellae and in this event are called Volkmann's canals.

3. *Lacunae and canaliculi:*

If now a somewhat greater degree of magnification is employed in the examination of these sections of bone it will be seen that the narrow channels which separate the lamellae are not of constant width. At fairly regular intervals dilatations which are usually fusiform occur having their greatest dimension parallel with the course of the interlamellar space. These dilatations are known as lacunae (Fig. 304) and in living bone contain the bone cells or corpuscles. Occasionally a lacuna may be formed in instead of between lamellae but this is not usual.

In order that the finer anatomy of these lacunae may be clearly seen it is necessary that they be filled with air or, better, impregnated with silver or with some dye such, for example, as fuchsin and studied in a very thin section mounted beneath a cover glass in balsam. In such a preparation it is at once evident that the lacunae are not simple spaces but have minute branching tubules radiating from them which perforate the lamellae in all directions. These branches (*the canaliculi*) (Fig. 304) from one lacuna intercommunicate freely with other canaliculi from the same lacuna and with canaliculi from other lacunae. They do not as a rule pass from one Haversian system to another although very occasionally this can happen. They do, however, open into the Haversian canal and into the space which surrounds the lamella as well as beneath the periosteum or into the medullary cavity. The canaliculi then form an intercommunicatory system of fine tubules which perforating the lamellae in all directions open into the interlamellar spaces, the lacunae, and on all surfaces of the bone. Fluid may pass by these channels from the Haversian canal to all the lamellae of the Haversian system to the medullary cavity or to the outer surface of the bone bathing every cell in the system as well as the osteoblasts which surround it.

4. *Structure of the lamellae:*

The finer structure of the lamella cannot be understood from a study of sections no matter how they may be prepared. As a matter of fact certain points about it are in dispute almost a century after Sharpey began its study with modern methods of microscopy. To attempt to gain any knowledge of the lamella from ground preparations of dried bone is like studying a coralline hydroid by examining a coral bead. In the first place one must recognize that the lamella *per se* is simply a connective tissue impregnated with salts, for the most part the tertiary phosphate of lime, and that like

all other connective tissues the lamella is a product of the activity of cells. Certainly the lamellae are chiefly made up of closely interwoven fibers which have the chemical characteristics of the white fibers of connective tissue. Many investigators believe that the fibers of the lamella are imbedded in a homogenous ground substance of a gelatinous nature. The fibers can best be seen at the edges of circumferential lamellae which have been pared from the subperiosteal surface of a decalcified bone. The majority of them run in straight lines but they may be wavy. They decussate and some pass from one lamella to the next, and they may cross each other so that in section some are cut longitudinally and some transversely. They are themselves subdivided and are made up of very fine fibrils. The spaces between lamellae are only apparent as they are bridged by the ground substance of the lamella in which the fibers are embedded. Furthermore the lamellae are bolted together with the perforating fibers of Sharpey. The latter are bundles of white fibers which can be seen in thin sections of decalcified bone or teased preparations to pass through a series of ground lamellae so that the lamellar systems may be thought of as threaded or strung on them. While most of these fiber bundles are composed of white fibers there are a small number of them which are of the yellow elastic variety.

The origin of these perforating fibers is from the periosteum and from the fibers of tendon bundles and from the fibers of the lamellae themselves. The bundles are broad at the base and taper to a point at their extremities. The white fiber bundles become calcified but the yellow elastic fibers never do and in preparations of bone from which the organic material has been removed the tubular canals which they have occupied can be seen. In the matrix described before salts of calcium and iron and certain other metals are laid down. It is generally held that the fibers of the lamella never become calcified but are embedded in the calcified ground substance.

II. HISTOLOGY OF DECALCIFIED BONE

It is only lately that the tremendous variability of the picture seen when sections of bones which have been decalcified are studied has been appreciated. The variation depends on the state of nutrition of the individual which is reflected in the activity of the cellular elements which take part in osteogenesis and osteoclasis, and is much more marked in the bones of young, than in adults or senescent animals. The most important factor in the determination of the histological picture is the abundance of the supply of the various substances which are necessary to the process of calcification viz. calcium, phosphorus, and the so-called anti-rachitic D vitamin. Secondary in importance in the determination of the condition of the bone are the other vitamins A.B.C., and the activity of at least one of the endocrine organs—the parathyroid gland. There may be more substances which

have a rôle in bone metabolism the importance of which we are not now cognizant of or at the most appreciate only imperfectly, and further we cannot be sure whether any of the above mentioned agents act directly by their presence in the tissue fluid which bathes the bone corpuscles, or indirectly through their pharmacological effect on the sympathetic neurons or blood vascular system. The author is, however, much inclined to the belief that all the changes observed in the bone tissue are produced indirectly in the manner aforesaid. Be this as it may, not only the minute but even the gross anatomy of the bones, especially in young animals, may be made to vary at will within wide limits by altering the metabolism of the animal through the composition of its food.

1. *Cellular structure:*

If a decalcified section be examined made from the bone of an animal kept on a diet which we have every reason to believe to be optimal for its

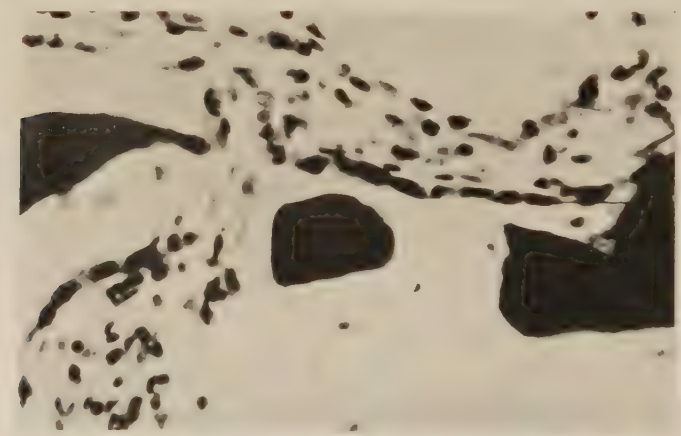


FIG. 307.—Osteoblasts of the pavement or flattened type: o, osteoblasts.

physiological well being the following facts may be observed. The lacunae are seen to be occupied by cells (bone corpuscles) with oval nuclei containing usually one nucleolus. The common shape of these cells is that of a spindle or oval but some are flattened and of greater breadth than thickness. If the proper technique has been used for coloring the sections the numerous processes which branch from the cytoplasm of these cells and traverse the canaliculi may be made to stand out sharply (Fig. 305). These processes are broader at the base and decrease in diameter as distance increases from the cell body. They anastomose freely with the processes from their fellows and often the point of junction between two processes is indicated by a tiny swelling or spherical or fusiform enlargement of the fiber. The medullary or the periosteal surfaces of the bone and its individual trabeculae are

covered or lined almost or quite completely by the same cells as those which are in the lacunae but in this position they are known as osteoblasts. The bone cells in this situation vary very much in their form. In the same bone, osteoblasts may be seen which have a tall cuboidal shape (Figs. 308–310). Osteoblasts surrounding other trabeculae may be so flattened that when a cross section through their bodies is examined only the long oval nucleus is seen distinctly, the cytoplasm being represented by a line (Fig. 307). Seen on the flat these cells have a polygonal shape and a relatively wide granular endoplasm about the nucleus, the ectoplasm being a narrow clear zone (Fig. 308). The bodies of the individual corpuscles forming the layer of osteoblasts are never quite in apposition since they are always separated by a narrow space so that a sheet of them has a mosaic-like appearance. With the aid of proper technique in staining, processes from these cells may be seen extending into the lamella from the side which is in contact

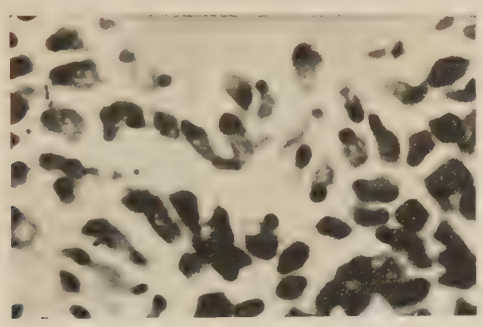


FIG. 308.—Flattened osteoblasts photographed on surface of bone from jaw of rat.

with the bone to anastomose with the like processes of their predecessors the bone corpuscle and with each other.

Hence just as the canaliculi form a system of tubules anastomosing and extending from the depth of the bone throughout its entire area to its surface so the above described processes of the bone cells in a lamellar system constitute a protoplasmic network by means of which each cell is in direct or indirect communication with all the other elements in the same system and with the osteoblasts on the surface of the bone. Moreover it is possible under certain conditions, as is stated below, to trace processes from the osteoblastic layer to cells of the same nature surrounding blood vessels in the medullary cavity and from them to a perivascular network of fibers which blacken with gold chloride. It is not unjustifiable to consider as a possibility a direct connection between the bone corpuscle and the vasomotor nerves about the vessels of the bone just as there is a direct circulation of the fluid bathing the cells in the lacunae through the system of

canaliculi. The importance of such a connection for the physiology of the bone is obvious.

2. *Lamellar structure:*

Little can be seen of the structure of the lamellae in completely decalcified sections because of the alterations in it caused by the decalcifying agents. Completely decalcified bone tissue is acidophilic but bone carefully decalcified, for example in Muller's fluid, is colored blue in preparations made with hematoxylin and eosin. In such preparations of growing bone a narrow acidophilic border 1-2 in breadth can be seen surrounding the calcified bone or lining the Haversian canals. This is the so-called "physio-

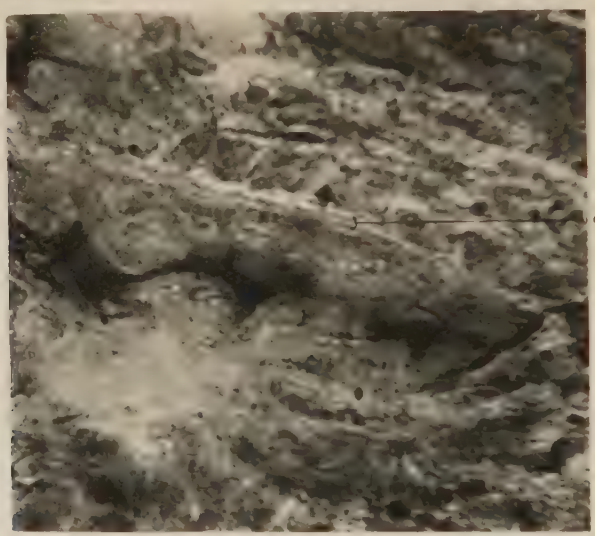


FIG. 309.—Visible osteoid from normal trabeculae: op, osteoid.

logical osteoid" (Fig. 309) in other words the newly formed lamellar tissue which has not had time to become calcified. In growing bone the trabeculae in the spongiosa contain in their centers, like a core, the spicule of calcified cartilage matrix about which the trabecula is formed. This structure is stained an intense blue in preparations stained with hematoxylin and has on either side deep bays directly opposing each other which form the walls of the cartilage areolae.

3. *Haversian system:*

The Haversian canals (Fig. 310) are like the medullary space lined with osteoblasts and filled with loose delicate reticular tissue. Blood vessels, usually an artery and one or more thin-walled veins run in them and lymphatic vessels may be found in the larger ones, while in the smaller, the

lymphatic system is represented by perivascular spaces. Vasomotor nerves are also of necessity present in them and the larger spaces contain marrow. Even in the smallest a few hematopoietic cells are present. In short the Haversian canal is a finger-like projection from the marrow cavity about which bone has been built and contains the same structures which are found in the *cavum medullare* itself from which the canal is a branch.

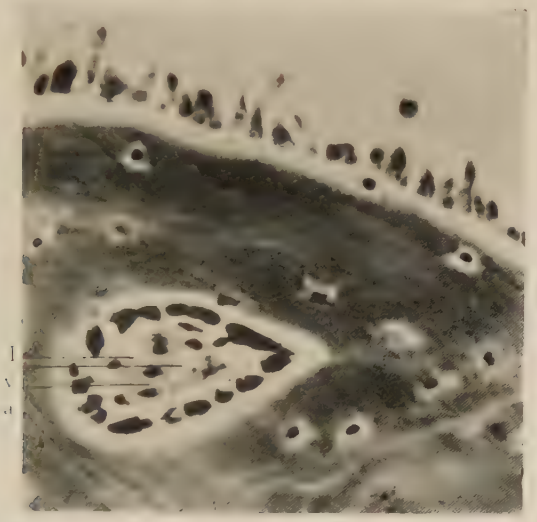


FIG. 310.—Haversian canal from a section of normal bone: I, lymphatic; a, artery; v, vein.

4. *Variations in the cytology of bone:*

In examining sections of bone from animals in different conditions of nutrition it is often difficult to determine a border-line between physiological and pathological states and of course in this tissue as in others pathological changes are often only exaggerations of physiological conditions.

The changes in the histological structure are in most cases foreshadowed by alterations in the gross architecture of the bone. The bone cell may be stimulated to take on two distinct forms of activity. To one type of stimulus the cell responds by an excessive production of osteoid or lamellar substance, an over production with which the process of calcification cannot keep pace. As a result of this activity the bones become thickened and on histological examination all trabeculae are found surrounded, as the Haversian systems are lined, with unusually broad zones of uncalcified lamellar tissue (Fig. 311). The osteoid corpuscles are small and flattened and are widely separated. The layer of osteoblasts which line the medullary spaces and Haversian canals are so flattened as to need careful search for their discovery. Corresponding changes take place in the cartilage and a

picture closely related to rickets is the result. On the other hand bones may be produced in which the reverse of the above is found (Fig. 312). The cortex is a lattice work of lamellae poorly calcified with wide marrow or Haversian spaces. The trabeculae of the cancellous tissue are very slender and very numerous. The lacunae are relatively enormous (Fig. 314) and are filled with huge bone corpuscles with spherical or oval nuclei so close together that one lacuna almost communicates with its neighbors. The layer of osteoblasts may be several cells deep and the osteoblasts themselves are large cells with round or oval nuclei with a faintly basophilic cytoplasm each containing a single large vacuole filled with acidophilic material lying on one side of the nucleus and in close approximation to it. Many free osteoblasts can be recognized lying singly and in groups about in the medullary cavity.

Such pictures are found in areas in which bone is either being built rapidly in large quantities as growing antlers of deer or where bone is being rapidly built up and as rapidly destroyed. Again, in bones in which osteogenesis and resorption are at a standstill, the bony tissue is present in small amounts (Fig. 313). The compacta is thin and slender, the trabeculae of the spongiosa are very few in number or almost entirely lacking and all lamellar tissue is densely calcified. In such bone groups of osteoblasts are found free in the medullary cavity having no relation to formed bone.

The precise significance of variations in bony structure such as those described above is not clear, nor are the mechanism of their production, and the nature of the stimuli which evoke these anatomical responses definitely known.

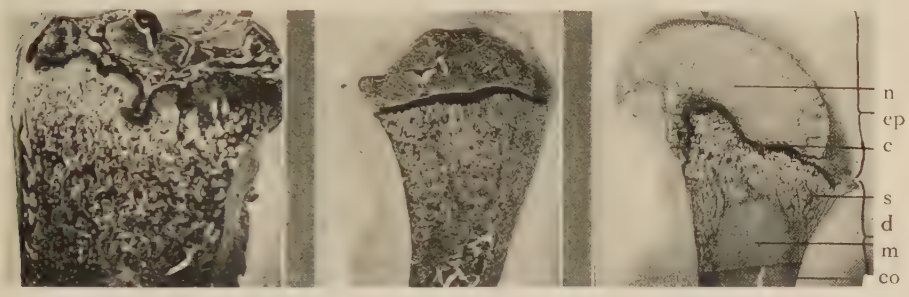
5. *Phenomena which accompany resorption of bone:*

For a long time the process by which previously formed bone is broken down during the normal process of growth has been regarded as settled, but of late some doubt has begun to be cast on this established tradition.

When the border of the bony trabeculae are examined with the aid of the higher powers of the microscope at some places along the periphery they may be seen to be indented by little bays called the *foveolae* or *lacunae of Howship* and in these bays are found large multinucleated masses of protoplasm which are known as *osteoclasts* (Fig. 315). These giant cells have a granular cytoplasm in which there often shows a clear border at the side of the cell which abuts on the bone. They may have ten or more nuclei which are placed closely together usually in a mass without definite arrangement. A phagocytic function has been assigned to these cells arbitrarily because of their position in the foveolae and because without doubt they are more numerous in bones undergoing rapid resorption. However, their power of phagocytosis is doubtful, and they do not occur in sufficient numbers to account for the rapidity with which absorption can take place

in growing bones under the influence of certain stimulae. They may play a part in the resorption of bone but it must be a relatively insignificant one. They are said, and I believe with reason, to be derived from the fusion of osteoblasts and have been thought to redivide to form these cells when their usefulness is done.

During resorption there is a vigorous invasion by small vessels from the marrow cavity of bone which is being resolved. These vessels grow into the disappearing bone in loops surrounded by a small amount of delicate reticular tissue just as the primary vascular loop invades the cartilage anlage of



311 312 313

FIG. 311.—Sections through the tibia of a rat fed on a diet which produces great thickness of the shaft of the bone. Compare with Figures 312 and 314. Note the great thickness of the trabeculae, the thickness of the cortex and the intense calcification.

FIG. 312. Section through a bone in which absorption and osteogenesis are proceeding with great rapidity. Note the enormous number of very fine trabeculae which are poorly calcified.

FIG. 313. Bone showing marked osteoporosis in which calcification and resorption are at a standstill. Note the lattice-like cortex and the scarce trabeculae in the spongiosa. Compare with Figures 311 and 312. n, the nucleus of ossification; c, epiphyseal disks of cartilage; s, spongiosa; co, cortex; ep, epiphysis; d, diaphysis.

the bone from the perichondrium. They may pass completely through a trabecula and are known as perforating vessels.

Finally in the vicinity of trabeculae which are undergoing resorption large numbers of mononuclear phagocytes gather in the medullary tissue. Their business is undetermined but that they have some connection with the process of resorption would seem likely. It is doubtful if they have any other purpose than that which they serve when gathered about foreign bodies or new growths—that of scavengers which gather the debris which results from the destruction of calcified tissue and change it to a state in which it will be harmless and useful to the organism as a whole.

By far the most striking phenomenon however which accompanies rapid destruction of bone is the behaviour of the bone corpuscles themselves. These cells enlarge greatly (Fig. 316) and develop large single vacuoles in the cytoplasm which often exceed the nucleus in size. They assume a

polygonal shape and the nucleus becomes spherical or oval and has a more open and a finer meshed chromatin network than when in the resting stage of their existence. Simultaneously the deposit of lime salts at the borders of the lacunae becomes granular and disappears leaving a margin of decalcified, osteoid, lamellar tissue; the dimensions of the lacunae enlarge and the bony tissue disintegrates more and more until it totally disappears leaving only a mass of free osteoblasts to show the place it formerly occupied. One cannot examine a bit of bone in this condition and escape the feeling that it is being rapidly dissolved and devoured and that the osteo-

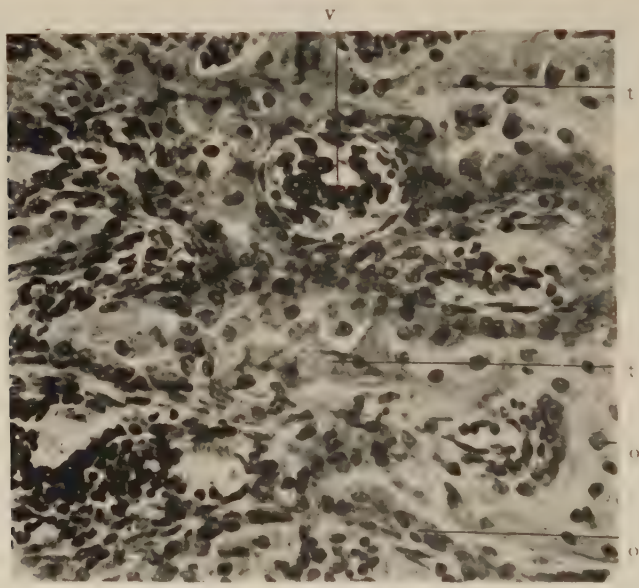


FIG. 314.—Section of a bone showing very intense osteoblastic activity. Resorption and osteogenesis are going on at an extraordinarily rapid rate: v, perforating vessel; t, trabeculae; o, osteoblasts.

blasts and bone corpuscles are responsible for its dissolution as they were for its construction. The author believes that the bone cell is an important factor in bone resorption but that it is such because of the assumption of such a function as phagocytosis, he feels to be hardly likely. All of the above described cellular and vascular phenomena occur when resorption is taking place, but the fact must not be overlooked that they may represent the results, and not the cause of the major change which is in progress. Resorption of bone is most probably a chemical process and may be carried on through the tissue fluids themselves. Cells of any sort can only act in the capacity of intermediaries and the vascular phenomena are probably no more than secondary.

6. Development of bone in the embryo:

The primitive skeleton of the embryo is as has been said above entirely cartilaginous (*embryonic cartilage*) but this condition is not of long duration. Almost at once the cartilaginous anlagen are complete, the cartilage corpuscles in the portion of the anlage which represents the definitive shaft of the bone begin to undergo the changes described above which precede preliminary calcification. Lime salts are then deposited in the matrix of the central portion of the shaft converting this area into calcified cartilage (Fig. 301). Each cell lies in a primary areola of calcified matrix. Coincident with this provisional calcification is the assumption of activity on the part of the embryonic periosteum. In this membrane which is disproportionately thick as compared with the size of the embryonic bone, osteoblasts develop from the undifferentiated mesenchyma and begin to deposit a layer of osteoid tissue in apposition to and surrounding the calcified cartilage of the shaft, which is almost immediately calcified. Thus a tube of bone is formed, flaring slightly at each end which is the beginning of the compacta of the cortex. Since osteogenesis began in the central portion, the long bone has thicker walls in this area, which become thinner as the ends are approached.

The formation of periosteal bone precedes the beginning of endochondral ossification, but not by much. Almost at once, the periosteal tube is pierced in the center at a single point and the calcified cartilage within is invaded by a knot of capillary blood vessels surrounded by a sheath of connective tissue derived from the periosteum. This vascular sprout pushes through the entire diameter of the shaft. The calcified matrix is removed from before it and primary marrow spaces are excavated which are called *secondary areolae*. These increase in size in all directions.

In a short time differentiation occurs in the capillary tuft which has invaded the calcified cartilage and the afferent and efferent capillary become respectively a primitive nutrient artery and vein. The tuft divides into two parts each of which develops into a cone-shaped mass of vessels the base being distal, the apex proximal to the center of the bone. The base which is perfectly flat is formed of a mass of young growing capillaries. As the apex is approached the number of vessels in the cone becomes fewer, and their diameter larger until they unite at the apex to form the nutrient vessels. In other words the blood enters through the nutrient artery which branches in two, one branch going toward either end of the bone. These secondary branches rapidly divide and subdivide to form a cone-shaped mass of vessels which terminate in capillaries. The efferent capillaries coalesce to form venules which are tributary to two main trunks, which in their turn unite to form the nutrient vein or veins.

As the nutrient tuft of vessels enters the bone anlage a delicate connective tissue is carried in with it derived from the embryonic periosteum.

This tissue, which is destined to be the marrow reeticulum, contains many primitive osteoblasts, and these begin the formation of endochondral bone. The young bone forming cells apply themselves to calcified spicules of intercellular matrix which have survived the ingrowth of the vessels and form osteoid tissue about them. This organic osteoid becomes calcified and becomes definite bone.

From this time the process of endochondral ossification is continuous unless interrupted by disease and is changed only in detail. The anlage is now represented by the terminal epiphyses of the bone, the central portion or shaft (diaphyses) having been replaced by spicules of endochondral and a tube of periosteal bone. The cartilaginous epiphyses of the bone grow

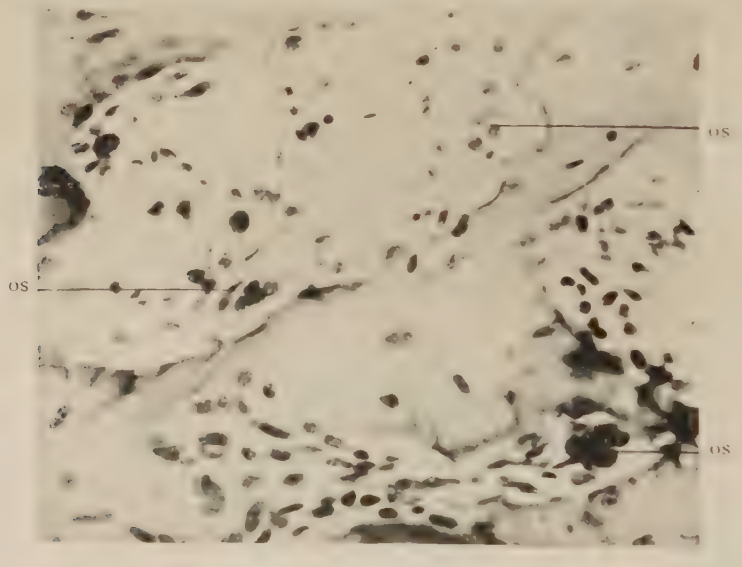


FIG. 315.—Osteoclasts in Howship's lacunae: os, osteoclasts.

with the growth of the organism in all dimensions. The growth of the capillary cone keeps just behind the growth of the cartilage and as the growth of the capillaries proceeds the calcified matrix of the provisional zone is continually encroached upon as a result of the onward march of these vessels and the tissue which surrounds them. The dormant cartilage cells of the provisional area of calcification lie in lacunae surrounded by calcified cartilage matrix. These lacunae are arranged in columns parallel to the long axis of the bone. As the vessels and connective tissue advance the lacunae are opened from their marrow side. The matrix between the columns of lacunae is spared destruction and remains as spicules which protrude into the marrow cavity between the advancing capillaries.

The agent which is active in the opening up of the cartilage arcola is not known. It may be as is generally supposed that the advancing vessel invades the arcola mechanically. It is, however, quite as likely that the actual entrance of the capillary into arcola is accomplished through the intervention of some of the accompanying cells—perhaps the young osteoblasts themselves—and that the opening of the arcola may be a chemical rather than a mechanical process.

The histological picture does little to help decide the question. The walls of the vessels are so delicate as to render their study difficult, and

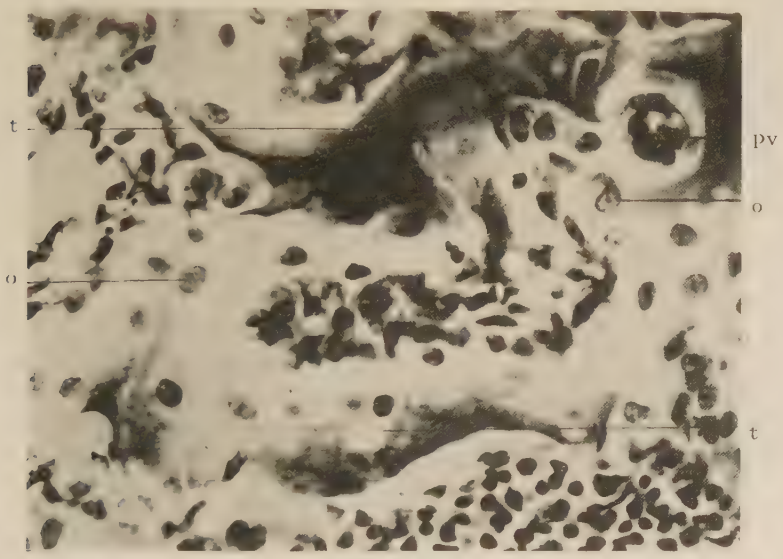


FIG. 316.—Resorption from a celloidin section of a bone of a rat: t, disintegrating trabeculae; o, osteoblasts; r, perforating vessel. Note that no osteoclasts appear in this picture.

often the only evidence of the presence of an invading capillary in a newly opened arcola is the blood cells which the vessel contains.

The fate of the cartilage cell released from its hibernation in the calcified matrix is still controversial. Some investigators believe that they disintegrate, others feel that some at least undergo a metamorphosis and are released to become osteoblasts and to play their part as such. Certainly in pathological bone, cartilage cells readily change into bone forming cells indistinguishable from those which have served this function from their beginnings, and all stages of transformation may be followed in histological sections. The same process may be seen to occur during the formation of the callus in the healing of fractures.

As the capillary vessels with their satellite osteoblasts and reticular tissue grow between the persistent spicules of cartilage matrix the osteoblasts apply themselves to the latter as to a trellis (Fig. 317) and the cartilage matrix serves on as a core to the spicule of spongious bone which is formed about it. If frozen sections are examined which were made from rickety bone stained by Ranvier's gold chloride method it may be seen that each capillary is surrounded by a lace-like tube made of osteoblasts each connected with its fellows by means of long slender processes which later will be enclosed in the canaliculi of the finished bone.

These newly apposed osteoblasts bury themselves in a material known as "*osteoid*" in other words organic lamellar tissue which is very rapidly calcified. The osteoid is the product of the osteoblast. In the formation of the osteoid however the processes of the osteoblast are not encroached on and their communication with their fellows is maintained through the canaliculi which remain open for that purpose.

Of the primary trabeculae which form the original spongiosa of the growing bone, a certain number grow larger and develop a lamellated structure because of the apposition of fresh layers of osteoblasts which build layer after layer of new bone. By far the greater number however have only a temporary existence and are destroyed almost at once as a marrow cavity is hollowed out by physiological resorption. The majority of those which remain eventually become arciform with their bases springing from the compact cortex and persist as the definite cancellous tissue. The shape of the individual trabecula of the cancellated spongiosa is determined by the lines of force developed by the direction of the stresses and strains to which the bone is subject.

Growth in length proceeds most rapidly at the ends of long bones. Growth in diameter is maintained by the constant apposition of fresh layers of periosteal osteoblasts, and the laying down by them of new lamellae of bone on the outer surface beneath the periosteum. Maintenance of sufficient size of the marrow cavity, is the result of progressive, probably intermittent resorption on the medullary surface, balanced to the requisite degree by the laying down of new bone lamellae. The latter process is the function of the osteoblasts which line the medullary cavity —the *endosteum*.

Since the form of the bones is constantly changing there is necessarily a constant flux of osteogenesis and resorption at different areas in the bone.

The cortex of the bone in the embryo is not a true compacta being to a certain extent cancellated and regular lamellation of the cortex occurs relatively late in the history of the bone. The Haversian systems develop as the result of deposition of concentric lamellae following the apposition of successive layers of osteoblasts about vessels which invade the cortex from the periosteum and the medullary cavity.

7. *Development of "membrane bones:"*

These probably represent the cutaneous bones of some of the lower forms of vertebrates. The development occurs in connective tissue membranes and is known as intermembranous ossification in contra distinction to "periosteal" and "endosteal" ossification. The perietal bone is a good example of a bone developed in this way. In a dense connective tissue membrane beneath the integuments of the skull, certain cells develop into osteoblasts and form an osteoid ground substance which becomes calcified. At the same time a change takes place in the fibers of the connective tissue. Straight radiating bundles are formed which are continuous with the ordinary fiber bundles at their extremities. Osteoblasts migrate along these fiber bundles depositing ground substance as they go, which becomes calcified to form true bone. The *osteogenic fibers*, so-called, are identical in composition with ordinary white fibers.

In this manner an embryonic bone is formed of irregular spicules radiating from a central mass. The spicules become joined to each other by branches and a lace-like network of bone is formed. The formation of osteogenic fiber bundles precedes the advancing growth of the spicules of bone. The union of the individual spicules by cross branches comes about in the following way. The osteogenic fiber bundles protruding from the ends of the spicules, radiate fanwise from the bone and the peripheral bundles from adjacent spicules come into contact so that when finally ossified, fusion is brought about between the adjacent bundles in circumscribed areas.

The interstices between the spicules are filled with connective tissue in which myeloid tissue develops and with blood vessels and osteoblasts. The interstices continually become narrowed by accretions of bone newly laid down on the old spicules.

In the meantime a periosteum has formed on either side of the cancellous tissue and the outer and inner tables of the fully developed bone are formed by this membrane. Growth continues in this manner until the bone comes into contact with its fellow on the opposite side of the skull.

8. *Calcification:*

Although the physiology of bone cannot be discussed here in detail this chapter would be incomplete without some reference to the facts which have lately come to light about the process of calcification. It may be said at once that while calcification is apparently one of the simplest of physiological processes, the study of it has been hedged about with difficulties which have caused the mechanism of it to be a matter of speculation until very lately. Even at present only that very small bit of the process which is subject to the most elementary laws of physics and chemistry is comprehended. Much and the most important part of the story can only be surmised. The actual precipitation of Ca_3PO_4 from the very dilute solutions

in which it is brought to the osteoid lamellae, and to the matrix of the cartilage probably is governed by simple laws of chemistry, but the mechanism by which the environment is controlled which makes the operation of these laws possible is most complex, and many apparently remote factors enter into it. It is essential in order that calcification take place that calcium and phosphorus should be present in the tissue fluid above a minimum concentration, but this minimum is a variable and is influenced by the other constituents of the fluid. The presence of any colloid, e.g. protein, delays or entirely inhibits calcification with a given amount of lime and

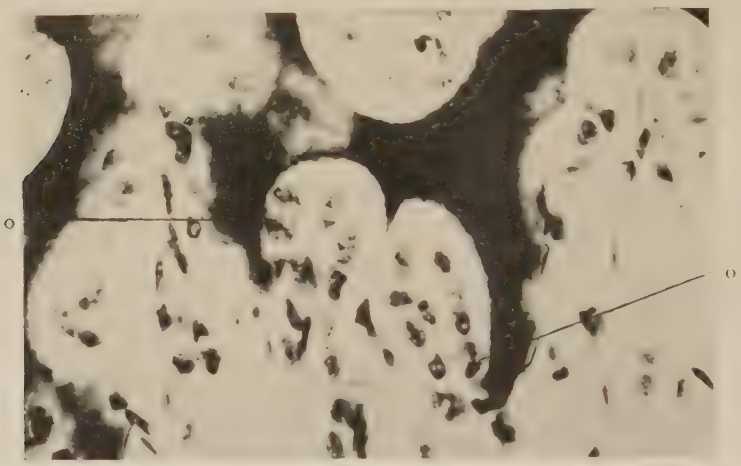


FIG. 317.—Section through the opened primary areolar at the epiphyseo-diaphyseal line of the tibia of a cat. The osteoblasts are approaching the spicules of cartilage matrix and applying themselves to it: o, osteoblasts.

phosphorus. If, however, the calcium and phosphorus concentration is increased, then calcification will go on in spite of the presence of an amount of colloid which would have inhibited it at a lower concentration of phosphorus and lime. Salts, especially those which are easily ionized, exert an inhibitory influence on the process, so that the increase in the concentration of these bodies, and of protein in the tissues of patients with chronic nephritis, may help to explain the failure of calcification which in children results in the chain of symptoms which have received the name "renal rickets."

9. *Halisteresis:*

In this connection mention should be made briefly of halisteresis. This name has been given the phenomenon of withdrawal from bone, circulation, and re-utilization of calcium salts,—long an hypothetical process, but now definitely known to take place. For years it was suspected that in the face

of a deficient supply of lime salts in the body they might be withdrawn from pre-existing loci of deposit, e.g. bone or teeth, and allowed to circulate in the blood as a protection to the central nervous system, or redeposited in new bone to insure maintenance of the growth of the skeleton. This is now known to be the case, and, moreover, it is certain that other mineral substances such as lead which may be deposited with the phosphate and carbonate of lime in the lamellar tissue, can be released, circulate and be redeposited in the skeleton or excreted.

III. BIBLIOGRAPHY

Albee, F. H. 1923. Fundamentals in bone transplantation. *J. Am. Med. Ass.*, **81**, 1429.

Bast, T. H. 1924. A note on the phagocytic activity of bone cells. *Anat. Record*, **28**, 91.

Drinker, C. K., Drinker, K. R., and Lund, C. C. 1922. The circulation of the mammalian bone-marrow. *Am. J. Physiol.*, **62**, 1.

Engelbach, W. and McMahon, A. 1924. Osseous development in endocrine disorders. *Endocrinology*, **8**, 1.

Haas, S. L. 1923. A study of the viability of bone after removal from the body. *Arch. Surg.*, **7**, 213.

Harris, H. A. 1926. The growth of the long bones in childhood; with special reference to certain bony striations of the metaphysis and to the rôle of vitamins. *Arch. Int. Med.*, **38**, 785.

Johnson, R. W. 1927. Physiological study of the blood supply of the diaphysis. *J. Bone and Joint. Surg.*, **9**, 153.

Mcewen, Sir W. 1923. The study of nature as shedding light on the structure and functions of man; the antler of the deer and its relation to the growth of bone. *Brit. Med. J.*, **2**, 91.

Macklin, C. C. 1918. Bone-repair in the rat vitally stained with trypan blue. *Anat. Record*, **14**, 43.

Miller, R. C. and Maynard, L. A. 1927. Calcification studies with rats fed different protein supplements. *Am. J. Physiol.*, **79**, 615.

Muller, G. L. 1917. Experimental bone marrow reactions. *J. Exper. Med.*, **48**, 399.

Parat, M. and Godin, M. R. 1925. Remarques cytologiques sur la constitution de la cellule cartilagineuse. *Compt. rend. Soc. de biol.*, **93**, 320.

Peabody, F. W. 1926. A study of hyperplasia of the bone marrow in man. *Am. J. Path.*, **2**, 487.

Proell, F. 1925. Beiträge zur vitalen Knochenfärbung. *Ztschr. f. Zellforsch. u. Mikr. Anat.*, **3**, 461.

Retterer, R. 1926. Technique nouvelle du tissue osseux. *Bull. d'histol. Appliq.*, **3**, 26.

Shipley, P. G. 1923. Faulty diet and its relation to the structure of bone. *Dental Summary* (Toledo), **42**, 137.

Shipley, P. G., and Holt, L. E. 1927. Effect of inorganic salts on calcification; preliminary note. *Johns Hopkins Hosp. Bull.*, **40**, 1.

Shipley, P. G., Kramer, B. and Howland, J. 1925. Calcification of rachitic bones in vitro. *Am. J. Dis. Child.*, **30**, 37.

Stump, C. W. 1924. The histogenesis of bone. *J. Anat.*, **59**, 146.

Tovernd, G. 1923. The influence of diet on teeth and bones. *J. Biol. Chem.*, **58**, 583.

Watt, J. C. 1923. The behavior of calcium phosphate and calcium carbonate (bone salts) precipitated in various media, with applications to bone formation. *Biol. Bull.*, **44**, 280.

Section XXI

THE SYNOVIAL MEMBRANE OF JOINTS AND BURSAE

CONTENTS

Section XXI

	PAGE
I. Distribution of the Synovial Membrane in Joints	738
II. Structure of the Synovial Membrane	738
1. Areolar type of synovial membrane	739
2. Fibrous type of synovial surface	740
3. Adipose type	741
III. Synovial Membrane Cells	742
1. Cytoplasmic constituents of synovial cells	744
2. Mitochondria	744
3. Intracellular fat	744
4. Golgi apparatus	745
5. Chromidial substances	745
IV. Structure of Synovial Surface as Revealed by Silver Nitrate Preparations	745
V. Synovial Folds and Villi	746
VI. Transition Zone between Synovial Membrane and Cartilage	748
VII. Blood Vessels of Synovial Membrane	749
VIII. Lymphatics of Synovial Membrane	750
IX. Nerves of Synovial Membrane	750
X. Normal Variations in Synovial Membrane	751
XI. Absorption from Joints	751
XII. Regeneration of Synovial Membrane	752
XIII. Synovial Fluid	753
XIV. Bursae and Tendon Sheaths	754
XV. Bibliography	755

Section XXI

THE SYNOVIAL MEMBRANE OF JOINTS AND BURSAE*

J. ALBERT KEY

PREVIOUS to the latter half of the eighteenth century the synovial membranes were believed to be the capsular ligaments of mucous (Haversian) glands, which secreted the synovial fluid. In 1763 Bonn described and named a continuous synovial membrane which covered all of the surfaces of the joint, and Bichat in 1800 noted that the joints were closed cavities and that the so-called Haversian glands were nothing more than pads of fat.

It having been demonstrated that the joint cavities were closed sacs, the question arose as to the character of the lining of these sacs. Were they mucous, serous or simple connective tissue surfaces? In the first half of the nineteenth century it was believed that the joints were lined by epithelium. The work of His on the primary germ layers caused the epithelial theory to be generally discarded and it was replaced by the theory that the joints were lined by mesothelium similar to that which lines the body cavities.

In 1866 Hueter used Recklinghausen's silver nitrate method on synovial surfaces and came to the then startling conclusion that joint cavities were not lined by a continuous layer of endothelial cells, but that the synovial surface was simply a rather cellular connective tissue surface and that the cellular lining was irregular and incomplete. The work of Hueter stimulated interest in the subject and led to considerable discussion as to the nature of the lining of joints. The older view that joints are lined by endothelium was supported by Schweigger-Seidel (1867), Landzert (1867), Steinberg (1874), Colomiotti (1876), Bentzen (1875), and Tillmans (1874 to 1876). Hueter's hypothesis was supported by Reyher (1874), van der Sluijs (1876), Herrman and Tourneux (1880), and especially in the masterly studies of Hagen-Torn (1882), Hammar (1894) and Braun (1894). A slight modification of Hueter's view that the synovial surface is a connective tissue surface was developed by Lubosch (1910) and supported by Segale (1913). These investigators hold that the synovial membrane is a specialized connective tissue surface in that it contains some cartilage.

The above literature has apparently escaped the notice of the authors of most textbooks on histology, and the synovial membranes are as a rule given scant attention or classed with the peritoneum, pericardium and pleura. This perhaps is one reason for the current opinion that joints and bursae are lined by endothelium, and why our knowledge of their structure is so meager. As a matter of fact, the synovial membranes differ from the body cavities structurally and embryologically. The primary body cavities arise early in embryonic life from the coelom, while the joints are developed much later as clefts which form by the liquefaction of cells in the center of the cellular articular discs of mesodermic tissue which has under-

*Figures in this section will be found on plates following bibliography.

gone more or less differentiation. The primary clefts are gradually enlarged by the movements of the joint.

I. DISTRIBUTION OF THE SYNOVIAL MEMBRANE IN JOINTS

The synovial membrane of a diarthrodial joint may be considered as a loose, flexible tube which is attached around the margins of the articular cartilages covering the ends of the bones which enter into the formation of the joint. The bone ends project for a variable distance into the tube and the synovial membrane covers the non-cartilaginous intraarticular portions of the bones and is reflected from them to line the fibrous capsule of the joint.

Thus the articular capsule consists of a fibrous or ligamentous layer which is lined by the *stratum synoviale*. These two layers are often so intimately fused that it is not possible to identify a definite synovial membrane. In other areas the synovial stratum is separated from the fibrous capsule by a wide layer of areolar or fat tissue. The synovial surface with the articular cartilages over the ends of the bones forms a closed sac. The fibrous capsule is in many joints incomplete and in certain areas the synovial membrane is supported by muscle or areolar tissue or rests upon the periosteum.

Certain joints are rendered more complex by the presence of interarticular ligaments, tendons or fibrocartilage. We shall consider the intraarticular ligaments and tendons as being covered by synovial membrane, although it should be pointed out that often no definite membrane exists on these structures and the surface layer differs very slightly from the underlying tissue. In the case of fibrocartilage which projects into the joint or forms part of the joint lining, we shall consider the synovial membrane as stopping at the margins of the fibrocartilage just as it does at the margins of the hyaline cartilage. It is, however, to be noted that this is a purely arbitrary division and that the surface zone of the fibrocartilage resembles the dense surface zone of the ligaments, tendons and certain other fibrous areas of the synovial membrane very closely. The same is true to a slightly less degree of the surface zone of the hyaline cartilage.

Thus in brief we shall consider the synovial membrane as covering all portions of the joint surface except those areas which are composed of cartilage.

II. STRUCTURE OF THE SYNOVIAL MEMBRANE

The lining surface of joints is a free connective tissue surface in which the cells are especially numerous. The term "synovial membrane" has been in general use so long that it is now fixed in the literature and we shall use it, but it is not a good term to use in speaking of synovial surfaces because in

many areas the structure of the surface is similar to that of underlying tissues and there is no definite demarcation of a layer which can be described as a synovial membrane.

While the general plan of the structure is the same in all synovial membranes, the character of the surface varies considerably in different parts of the same joint, in different joints of the same animal and in the joints of different species. These variations in structure seem to be adaptations of the surface of a particular area to the mechanical conditions to which that area is subjected. Hagen-Torn, Hammar and others have described "cell-poor" and "cell-rich" types of synovial membrane. To me it seems more reasonable to use the underlying tissue as a basis for classification, because it is this which determines the character of the surface. We shall describe three main types of synovial surfaces: (1) the areolar type, (2) the fibrous type and (3) the adipose type. But it is to be pointed out that these three types are not sharply defined or limited to any special region of the joint, but merge one into the other, and that all transitions exist between them.

1. *The areolar type of synovial membrane (Figs. 318–322):*

This membrane covers those parts of the joint surface which are not subjected to pressure or strain and where function of the joint causes movement of the surface on the underlying tissue. This may be regarded as the typical synovial membrane and consists of a well-defined surface layer supported by loose areolar tissue which separates it from the underlying more robust structures of the joint capsule. In the knee joint of the rabbit it lines the quadriceps bursa, covers the sides of the femur, lines much of the lateral capsule and those portions of the posterior capsule which do not rest upon fat.

The surface layer is a thin sheet of collagenic fibrous tissue in which are embedded the synovial cells. The cells vary in number and in form in different areas. In the rabbit, the surface zone contains one or two layers of flattened endothelial-like cells which lie parallel with the surface. In some areas the cells are few in number and form a single thin layer, while in an adjacent area they may be much more numerous and arranged in three or more irregular layers. In such cellular areas the cells tend to be less flattened and may be cuboidal, oval or spherical in form (Fig. 322).

The cells do not lie on the surface, as does endothelium or epithelium, but are imbedded in the fibrous matrix and seem to lie indiscriminately on the surface, partly on the surface, or entirely beneath the surface (Fig. 320). It seems to be characteristic that the edges of the cells do not coincide with those of surrounding cells but may overlap or fail to meet, so that in cross sections the surface may appear to be devoid of cells for a distance of 50μ or more. Often a cell, the body of which lies deep in the fibrous matrix, sends a long, thick protoplasmic process up to the surface. The process usually lies

along the surface but may project into the joint cavity. In most areas the cells predominate, but in occasional areas the surface zone is largely collagenic, and here the cells may be in definite lacunae and resemble the cells of cartilage except that no nests of two or more cells are seen.

The fibrous groundwork is composed largely of delicate collagenic fibers which run in all directions and form a rather dense reticulum. In areas where the surface zone is subject to strain the fibers are somewhat coarser and tend to run in a direction parallel with the strain. Occasional fine elastic fibers are also present in the surface layer. The thickness of the compact surface zone may vary widely at different points in the same section. The simplest structure is a thin, delicate collagenic membrane, 2μ to 4μ in thickness, which is sparsely populated by endothelial-like cells (Fig. 319). From this simple form all gradations are met with up to a surface layer 20μ to 30μ in thickness, which may be either very cellular or fibrous in character (Fig. 319).

In addition to the synovial membrane cells, there are a few macrophages (clasmatocytes and monocytes), leucocytes and small round cells scattered here and there in the loose areas of the joint surface. These wandering cells are usually seen in the subsynovial areolar tissue but may be in the compact surface zone or they may penetrate into the joint cavity.

Beneath the compact surface zone is a layer of loose areolar tissue. This is composed of a coarse network of delicate collagenic and elastic fibers and large tissue spaces. It is sparsely populated by connective tissue cells and contains a variable amount of fat and numerous blood and lymph vessels.

The areolar layer is thickest in those areas where the surface moves most with function of the joint, as at the upper end of the quadriceps pouch, where it is continuous with the intermuscular areolar tissue of the thigh. In areas where the surface is subjected to more pressure and less movement it is much thinner or disappears entirely, and the surface is of the fibrous type. The areolar tissue fills in the space between the synovial surface zone and the fibrous capsule or other tissue surrounding the joint. Over the areas where the joint is surrounded by muscle it fills in the spaces between the muscle fibers and the surface zone, and here it may be very irregular in thickness, as some of the muscle fibers may be very close to the surface, while others are separated from it by a wide zone of areolar tissue.

2. *The fibrous type of synovial surface (Figs. 324 and 325):*

This covers the interarticular ligaments and tendons and lines those portions of the joint which are subjected to pressure or strain. It is characterized by the dense collagenic tissue of the surface zone and usually by the fact that the surface zone differs from the subsynovial tissues only in that it is more cellular and that the cells are slightly larger and are arranged parallel to the surface. In the rabbit's knee joint it covers the interarticular

ligaments, the interarticular tendons, portions of the lateral capsule which are lateral to the quadriceps tendon, the anterior portions of the sides of the femoral condyles and areas of the surface where the dense fibrous capsule or lateral ligaments are not separated from the synovial surface by areolar or fatty tissue (Fig. 326).

This corresponds to the "cell-poor" type of Hagen-Torn and Hammar, and the synovial surface is really composed of dense collagenic tissue which contains a variable number of cells.

As a rule the cells are few in number (Fig. 324), but in certain fibrous areas they are quite numerous, and here one finds three or four irregular layers of cells which lie quite close together. On the whole the cells tend to be well imbedded in the connective tissue surface, and often they are covered by a dense layer of collagenic tissue, but in some instances the cells appear to lie on the surface or are only partly covered by the fibrous tissue. In tendons and ligaments the surface cells are usually arranged in groups of three or more which lie with their long axes parallel with the collagenic fibers. In other fibrous areas the cells are scattered irregularly over the surface and have no definite arrangement. For the most part the cells are quite widely separated and in certain areas appear to be definitely encapsulated (Fig. 325). Hammar has described broad outer and thin refractile inner capsules around many of the cells in his cell-poor areas. I am inclined to regard these encapsulated cells as cartilage cells or transition stages between synovial membrane and cartilage cells. It is to be noted that unusual pressure may cause the development of fibrocartilage in the synovial surface. In my experience the encapsulated cells have been largely limited to the transition zones between the synovial membrane and the cartilage or to areas which were definitely cartilaginous.

The matrix of the fibrous type of synovial surface is composed almost exclusively of coarse collagenic fibers which lie parallel with the surface and pursue a wavy course. The surface fibers are usually smaller in diameter than are the deeper fibers and they may be composed of loose bundles of fibrillae. The fibrous tissue of the surface merges with that of the deeper structures and there is no subsynovial areolar layer. For the most part the coarse fibers lie parallel, but there are a moderate number of fine cross fibers which run in all directions and bind the main fibers together. There are also a few fine elastic fibers scattered through the fibrous matrix.

3. *Adipose type:*

This is the form of synovial membrane which covers the fat pads which project into the joint cavity. In the knee joint of the rabbit the largest fat pad is the prepatellar fat pad or the alar ligaments, situated in the space between the patellar tendon, the upper end of the tibia and the femoral condyles. A smaller fat pad lies between the upper end of the patella and

the cartilage of the quadriceps tendon, and others project into the posterior capsule.

The synovial membrane of the fat pads is similar to the areolar type described above, but the subsynovial areolar layer is absent and the membrane rests directly upon the adipose tissue. It resembles mesothelium more closely than do either of the types described above. In cross sections it is seen to consist of a single layer of cells which at first appear to rest directly upon the underlying fat cells. More careful examination, however, reveals a very thin collagenic membrane which supports the synovial cells and envelops the fat pad. The cells may lie on the surface of this membrane or may be partly covered by it.

As a rule the cells are arranged in a single layer and are so thin that it is often impossible to determine whether a given area contains a layer of protoplasm or is composed entirely of fibrous tissue. In other areas the cells are more numerous and may be oval or cuboid in form, and occasionally three or four irregular layers of such cells are present. In certain areas in the posterior capsule and at the apices of the fat pads there is a definite surface layer of loose fibrous tissue 15μ to 20μ in thickness, and here the cells are less flattened and more numerous. There may be from two to four irregular layers of cells imbedded in the loose fibrous tissue or it may contain only a single layer of spindle-like cells which tend to lie very close to, if not actually on, the surface.

The fat cells are always separated from the surface by a definite layer of collagenic tissue and synovial cells, and the synovial cells which cover the fat pads show no greater tendency to accumulate fat than do those of other parts of the synovial surfaces. Some authors have described fat tissue as composing part of the joint surface, but I believe that these were instances in which the normal surface zone had been removed by trauma, or of sections incorrectly oriented.

III. THE SYNOVIAL MEMBRANE CELLS

The synovial membrane cells are fixed connective tissue cells which, because of their position on or near the synovial surface, have become slightly modified in form and perhaps also in function. As a rule they are slightly larger and their nuclei stain more deeply than do those of the cells of the subsynovial tissues, but often there is so little difference between the surface cells and those of the deeper tissues that it is not possible to differentiate them except by their position and arrangement.

As seen in cross sections of the joint surface, the synovial membrane cells can be divided into three types: (1) long, thin spindle-like cells; (2) short, thick spindle- or comma-like cells, and (3) ovoid or spherical cells. The types mentioned are not, however, to be considered as different varie-

ties of cells, but are merely different forms of the same kind of cell, the form being determined largely by the situation of the cell.

The long spindle-like cells are found in the membrane over the fat pads and in the thin areas of the areolar type of joint surface. They are really flattened endothelial-like cells which may have a very irregularly shaped cell body with several short blunt processes, or the cell body may be a fairly regular round or oval disc-like structure. The nuclei are thin, flattened and usually stain deeply, but can be shown to possess a coarse chromatin net.

The short spindle-like or comma-shaped cells, as a rule, are found in the rather dense areas of the surface or in the more cellular areas of the areolar type and especially on the villi. The cell body is usually flattened in a direction roughly parallel with the surface and it often terminates in one or two thick protoplasmic processes which may be of considerable length. The rather large oval nuclei stain deeply and contain a coarse chromatin network.

The spherical or ovoid cells are commonly found in those areas where the synovial membrane contains two or more layers of cells which lie quite close together. They are usually mingled with the short spindle- or comma-shaped cells and the two types are not clearly defined, as all gradations exist between them. These cells, too, may possess protoplasmic processes.

The protoplasmic processes of the synovial cells are not visible in ordinary preparations, but in thin sections which are well stained in hematoxylin and eosin or lightly stained in Mallory's phosphotungstic hematoxylin, a surprising number of the synovial cells are seen to possess one or more processes which may or may not be branched (Fig. 328), and occasionally one finds areas in which the joint surface appears to be formed by a rather dense network of these processes. Braun (1894) and others have described these protoplasmic processes as anastomosing with one another, but my observations lead me to believe that this is apparent rather than real and that no syncytium is present.

Hammar (1894) and others have described at length the various forms of nuclei which may be encountered in the synovial cells. They have described pycnotic nuclei, branched nuclei, triangular nuclei, spiral and curved nuclei and other forms. To my mind, all of these are senile changes or mechanical alterations of the nuclei of the usual synovial cell, which is essentially the same as that of the ordinary fixed connective tissue cell.

In small animals practically all of the synovial cells contain only one nucleus, but in larger animals and particularly in joints in which arthritic changes exist, a variable number of the synovial cells contain two or even three nuclei. Mitotic figures are rare in the synovial cells, but I have twice seen a mitotic figure in a synovial cell in the normal knee joint of an adult dog.

1. *Cytoplasmic constituents of the synovial cells:*

In ordinary Zenker or formalin-fixed material stained with hematoxylin and eosin or any of the other common stains, the cytoplasm of the synovial cells is pale and homogeneous and stains faintly with acid dyes. It contains no granules and very rarely a cell is seen to contain one or two rather large clear vacuoles. I have seen these vacuoles in preparations fixed and stained by many methods, but have not been able to stain their contents.

If the living cells be examined in isotonic salt solution, the cytoplasm has the appearance of finely ground glass, and a greater percentage of the cells are seen to contain one or two clear vacuoles, but no definite granules are visible. If now the cells be stained supravitally with neutral red, it will be found that the vacuoles remain clear and that the cells do not contain any specific granules or vacuoles which stain with the neutral red.

2. *Mitochondria:*

The mitochondria are so minute that they can be stained only with difficulty in the fixed preparations and are not visible in the living unstained cell. They can, however, be readily demonstrated by staining the living cells supravitally with a dilute solution of Janus green B (1 to 10,000 in isotonic saline). In such preparations the mitochondria appear as very minute green granules which are scattered diffusely through the cytoplasm. Very rarely a short green rod may be seen; but, in my experience, there have been no long rods, spherules or thread-like forms. The mitochondria may be more numerous in one area of the cytoplasm than in another, but they exhibit no constant tendency to be clumped around the nucleus or in any other region of the cell, and the protoplasmic processes contain mitochondria in about the same proportion as does the protoplasm of the cell body. They are more abundant in the larger cells, but are not very numerous in any of the synovial cells. In joints which have been subjected to irritation or infection, the synovial cells are increased in size and contain more mitochondria than normal, but I have noted no changes in form and no abnormal increase in the concentration of the mitochondria in the cytoplasm of such cells.

3. *Intracellular fat:*

In tissue fixed in osmic acid, an occasional cell in the synovial membrane is seen to contain from one to five minute black granules. These cells may be either synovial membrane cells or macrophages. However, the great majority of the synovial membrane cells contain no demonstrable fat, and those which cover the fat pads exhibit no greater tendency to contain intracellular fat than do those of other areas.

4. Golgi apparatus:

Neither the Golgi apparatus nor its clear image—the canalicular apparatus—has been demonstrated in the synovial membrane cells. I have examined material which has been fixed in osmic acid over a period of four weeks and have found no trace of a Golgi net in any of the cells in the synovial membrane.

5. Chromidial substance:

There is no evidence in the literature that the synovial cells contain any chromidial substance and I have not been able to demonstrate it in any of my preparations. Apparently the cytoplasm is optically uniform throughout the cell and contains only a moderate number of minute mitochondria. An occasional cell contains one or more clear vacuoles and very rarely a cell contains a few minute fat droplets.

IV. THE STRUCTURE OF THE SYNOVIAL SURFACE AS REVEALED BY SILVER NITRATE PREPARATIONS

Hueter (1866) used the silver nitrate method of von Recklinghausen in his studies on the structure of synovial membranes and believed that it revealed the true structure of the surface. Tillmanns (1874 to 1876), Schweigger-Seidel (1867) and others held that the pictures obtained by silver nitrate impregnation were artefacts produced by the coagulated synovial fluid on the surface. Reyher (1874) and other investigators have, however, demonstrated that the clear spaces in the silver preparations do really represent cells.

Just as there is considerable variation in the structure of different areas of the synovial membrane, so do the pictures obtained by the silver nitrate method vary greatly. Figure 329 is a photomicrograph of a silver nitrate preparation from the lateral capsule of the knee joint of a rabbit. The nuclei are not stained. It is fairly representative of the average structure of the loose areolar type of synovial membrane. It is to be noted that the cell outlines are very irregular and that the intercellular substance, as represented by the black lines, varies greatly in thickness. In other words, the cell borders often fail to coincide and the picture is very different from that of an endothelial surface.

There are also to be seen many very small, circumscribed clear areas which are obviously too small to be cells. These were believed by the older investigators to represent stomata which communicated with the lymph channels beneath the surface. From what has been said above of the structure of the synovial membrane, these small areas probably represent the protoplasmic processes of deeper cells which project into the layer included in the photograph.

A very different type of synovial surface is pictured in Figure 330. It is a similar preparation from the dense fibrous type of synovial membrane bordering the fibrocartilage of the quadriceps tendon in the knee of a rabbit. It is to be observed that the cell spaces are smaller, much more widely spaced and that the short processes are more clearly shown. The dark area represents the fibrous matrix and the vague spots represent cells at a different focus.

The two figures represent extreme types of the synovial surface as revealed by silver nitrate. In Figure 329 the surface is largely cellular, and in Figure 330 the intercellular substance predominates. While most of the surface gives pictures resembling that illustrated in Figure 329, it is to be noted that various intermediate grades of cellularity and intercellular substance are to be found and that one type shades gradually into the other.

The question naturally arises as to whether these pictures actually represent the true outlines of cells on the synovial surface. They do not. As Hammar (1894) has pointed out, the finer processes of the cells are not revealed by this method, but can be demonstrated in especially good hematoxylin and eosin stains. Furthermore, it is not possible to prevent the silver nitrate from penetrating the surface, and consequently the method simply gives a rough cross section of the cell bodies in any given area, but is not reliable in determining whether or not any given cell is on the surface or is covered by a layer of fibrous tissue. It is probable that most of the cells pictured in Figure 330 lie beneath the surface and it is also probable that many of the cells illustrated in Figure 329 are more or less covered by fibrous tissue.

V. SYNOVIAL FOLDS AND VILLI

A variable number of folds and villi project from the inner surface of the synovial membrane of most joints. They vary in size from microscopic projections to large, well-defined anatomical structures, such as the fat pads (alar ligaments) of the knee joint.

The folds may be transitory, due to the looseness of the synovial membrane in regions where there is a thick subsynovial layer of areolar tissue which permits considerable movement of the synovial surface with joint motion. These folds appear and disappear with joint motion and are simply projections of the areolar type of membrane into the joint cavity and present no special structure. Or they may be permanent structures either in the form of membranes spanning portions of the joint or as definite projections of the surface.

The folds which span portions of the cavity are simply sheets of areolar or loose fibrous tissue, both surfaces of which are formed by synovial membrane, and they have a free edge which is, as a rule, fairly regular in contour. The synovial surfaces may be of the areolar or fibrous type.

The marginal folds around the articular cartilages of many joints are definite outgrowths of the joint surface and are covered by synovial membrane which is usually more cellular than is that in the immediate vicinity (Fig. 331). The free edge is, as a rule, very irregular in contour and may be studded with villi. The subsynovial portion of the marginal folds is composed of vascular areolar tissue. In the thicker folds there is a variable amount of fat and dense fibrous tissue.

The villi are circumscribed outgrowths of the synovial surface. They vary directly in size and number with the size of the joint. In the joints of small animals, such as the rabbit, the synovial surface is, for the most part, smooth, and only a few microscopic villi can be found in the joint recesses; while in the larger joints of man and the large domestic animals, the entire synovial surface may be studded with macroscopic villi and some of these may be over 2 cm. long and 0.5 cm. thick. The villi may be pedunculated or sessile in type and vary greatly in shape and structure. The larger ones may have secondary villi projecting from various points on their surface (Fig. 332).

The structure of the villi, as a rule, corresponds to that of the joint surface to which they are attached. That is to say, villi springing from areolar areas are areolar in structure, villi springing from fibrous areas are fibrous in structure and villi springing from fat areas contain considerable fat.

The simplest form of villus is a small projection of collagenic fibrous tissue into the joint, and such a villus may be entirely devoid of cells and blood vessels. The collagenic fibers run lengthwise of the villus and pass down into the subsynovial tissues. Slightly larger fibrous villi may contain a variable number of cells which are similar to the cells of the surrounding fibrous tissue. Such villi are usually club-shaped, and the cells are more numerous near the free end and may lie on the surface or beneath the surface of the villus (Fig. 333). Large villi springing from fibrous areas contain a few blood and lymph vessels and are more cellular than the surrounding synovial membrane.

On an areolar area of the synovial membrane the simplest type of villus is a heaping-up of the surface cells until they project into the joint. A slightly larger form is a minute teat-like projection which contains a few small blood vessels and some areolar tissue. These small villi are quite cellular and the synovial cells form a fairly complete surface layer and are also scattered indiscriminately through the substance of the villus. The cells of these villi are usually larger and more branched than the cells of the adjacent synovial surface. In the larger villi of the areolar type there are numerous blood vessels running in the long axis of the villus and there is a considerable amount of collagenic fibrous tissue in the central portion (Fig. 334). Elastic fibers are less abundant in the villi than they are in the surrounding synovial membrane. The larger villi also contain a variable amount of fat.

The villi which arise from the free margins of the fat pad resemble those described above, except that they are less vascular and usually contain more fat cells and collagenic fibers. There also occur simple projections of the fat-pad surface with a thin covering membrane and a core of fat cells with few or no blood or lymph vessels. These might be classed as villi or as irregularities in the surface of the fat pads.

VI. THE TRANSITION ZONE BETWEEN SYNOVIAL MEMBRANE AND CARTILAGE

At the points where the joint surface changes from a synovial to a cartilaginous surface, the synovial membrane is more or less modified in structure. This modified synovial membrane is known as the transition zone. Around the hyaline cartilage, the transition zone may be described as a wedge-shaped process of synovial membrane which covers the free surface of the margins of the cartilage. The base of the wedge coincides roughly with the margin of the cartilage and the thin edge extends for a short distance over the cartilage surface and gradually merges with it. The synovial membrane of the transition zone is of the fibrous type and the character of the surface gradually changes from this to that of the cartilage. In this zone the cells are for the most part beneath the fibrous surface and at the base of the wedge are quite numerous, three to five irregular layers of cells being present here, but in the thin edge of the wedge the cells progressively decrease in number and size.

The change from synovial membrane cells to cartilage cells is a gradual transition. At the base of the wedge the cells are synovial in type, and as the cartilage is approached they become enclosed in lacunae and lose their processes. Transition stages may be described as isolated cells which are enclosed in lacunae and yet possess one or more short processes.

The subsynovial areolar tissue stops abruptly at the margin of the cartilage and is replaced by dense fibrous tissue which merges into the hyaline matrix. This areolar tissue is usually thickened around the margin of the cartilage and often folds or villi project from the synovial surface and overhang the transition zone. It is to be noted that these marginal folds and villi are outgrowths of the synovial membrane and not of the transition zone.

At the margin of the fibrocartilage, as present in the quadriceps tendon of the rabbit and dog, the transition zone is much wider than that described above. The tendon is surrounded by a dense fibrous synovial surface which gradually changes to the loose areolar type near the margins of the joint. This fibrous area of the synovial membrane gradually thickens as one approaches the tendon and the deeper cells are enclosed in lacunae and assume the characters of the cells of fibrocartilage. The superficial cells

become flattened, decrease in number and size, and lie beneath the surface to resemble the cells near the surface of the fibrocartilage. So gradual is the transition that it is not possible to determine where the fibrous synovial membrane ends and the fibrocartilage begins.

In areas such as the borders of the semilunar cartilages where fibrocartilage is surrounded by the areolar type of synovial membrane there is an abrupt change from the areolar to the fibrous type of synovial membrane, and the transition zone resembles that of the hyaline cartilage (Fig. 331). The wedge of fibrous synovial membrane extends for a short distance over the margin of the cartilage and gradually changes to the typical surface zone of fibrocartilage. Here, too, as around the hyaline cartilage, the subsynovial areolar tissue may be thickened and marginal folds or villi may be present.

The surface zone of fibrocartilage is so similar to that of the fibrous type of synovial membrane that there is considerable justification for including the fibrocartilages as part of the fibrous type of synovial surface.

VII. THE BLOOD VESSELS OF THE SYNOVIAL MEMBRANE

The synovial membrane is for the most part quite richly supplied with blood vessels which penetrate the fibrous capsule and, running parallel with the surface, form an intricate network in the subsynovial areolar tissue. Many of the smaller arterioles, venules and capillaries lie in the compact surface layer and may be separated from the surface only by an extremely thin layer of collagenic tissue, but they are usually covered by one or more layers of synovial membrane cells.

In silver nitrate preparations the superficial vessels appear as clear streaks across the field and the synovial cells appear to cease abruptly at the vessel wall, while the outlines of the endothelium of the vessel are often well shown. In other words, the vessels appear to lie naked on the surface, and this was the view by Hueter (1866) and other investigators. This peculiar action of silver nitrate has not been explained, but a study of a large number of sections convinces one that very rarely, if ever, does a vessel lie naked on the surface.

The blood vessels penetrate all but the smallest villi and many of the larger villi are extremely vascular. The vascular area of the synovial membrane is roughly the portion of the joint lined by the areolar type of synovial membrane. The circulus vasculosus articuli of Hunter should be mentioned. It corresponds to the transition zone around the articular cartilage over the ends of the bones and here the vessels are unusually abundant and form loops from which short end capillaries project into the cartilage margin.

The synovial membrane over the fat pads is only moderately vascular and the dense fibrous areas of the joint surface may be moderately vascular

or they may be very sparsely supplied with a few small vessels which are widely separated.

VIII. THE LYMPHATICS OF THE SYNOVIAL MEMBRANE

The arrangement and distribution of the lymph vessels of the synovial membranes are but imperfectly understood. The investigation is difficult because fluids injected into the joint under pressure or forced out into the subsynovial tissues by the pumping action of joint movements do not enter the lymphatics but diffuse through the subsynovial tissues, and in the smaller laboratory animals the lymph vessels are so small that direct injection is not practical.

Tillmanns (1876) was able to inject the lymphatics of the joints of the horse and the cow. He described two networks of lymph vessels: (*a*) a very rich and intricate network of wide lymph vessels which lay just beneath the synovial membrane, and (*b*) a deeper network of large lymph vessels which anastomosed with one another by means of extremely fine lymph capillaries. The lymphatics were much more numerous than the blood vessels and the superficial network was covered by the finest blood capillaries but lay between the synovial surface and the small arterioles and venules of the subsynovial tissue.

Subsequent investigators have, however, questioned the above findings on the grounds that Tillmanns injected tissue spaces rather than lymphatics. Magnus (1923), using the hydrogen peroxide method, described a superficial subendothelial network of wide lymph spaces with strongly curved contours and a deeper system of parallel vessels.

The question of whether or not the joint cavity communicates directly with the lymphatics by means of stomata has been answered in the affirmative by Mosengeil (1876) and in the negative by Hagen-Torn (1882), Braun (1894), Kroh (1904) and Noetzel (1906), while Hueter (1866 to 1867), Tillmanns (1876) and Riedel (1880) leave the question open. In my own studies on synovial membranes I have seen no evidence that such stomata exist. The silver nitrate preparations often contain areas which appear to be stomata, but as I have noted above, silver nitrate preparations of the synovial surface are very unreliable and must be interpreted with care.

IX. THE NERVES OF THE SYNOVIAL MEMBRANE

Hagen-Torn (1882) described nerve trunks which pierced the fibrous capsule with the larger vessels and accompanied the vessels to the subsynovial tissues where they sent off small branches to anastomose with the branches of similar nerves on all sides. The nerves contained both medullated and non-medullated fibers. The fibers did not penetrate the surface layer of the membrane or enter the villi.

The nerves ended in various ways: some formed extensive terminal arborizations, some broke up into two to four terminal branches and others ended in bulbs or long plates. Pacinian corpuscles were present in the synovial tissues of all joints and varied greatly in size and form in the joints of different species.

X. THE NORMAL VARIATIONS IN THE SYNOVIAL MEMBRANE

The description of the synovial membrane as given above is drawn largely from the author's studies on the joints of rabbits and dogs. The variations in the structure were noted and were roughly correlated with the function of the particular area involved. In man, and in the larger domestic animals, the structure of the synovial membrane is similar to that of the smaller animals, but is formed on a larger scale—the synovial cells are, on the average, larger and are more numerous, the fibrous matrix is more robust and the synovial folds and villi are greatly increased in size and in number.

Not only does the synovial membrane vary in different joints and in different species, but it varies in the same joint in the same species in different age periods. The senile changes in joints have been especially studied by Weichselbaum (1877) and Heine (1926). Weichselbaum noted both progressive and regressive changes in the synovial membrane in aged individuals. The progressive changes consisted of increase in the number of cells, increase in the size and number of villi and a tendency for islets of cartilage to be formed by metaplasia of the synovial membrane cells. The regressive changes consisted of fatty and amyloid degeneration of the cells and degeneration of the connective tissue ground substance of the synovial membrane.

So marked are the variations in structure incident to old age that often it is not possible to determine whether the changes noted in a given joint are senile in character or due to arthritis deformans (hypertrophic arthritis).

XI. ABSORPTION FROM JOINTS

Mosengeil (1876), Braun (1894), Jaffe (1897), and Key (1926) have studied the methods by which solutions, colloids and suspensions of particulate matter are absorbed from joint cavities. I have found that true solutions and fine colloids such as an aqueous solution of trypan blue diffused through the synovial membrane and passed out into the subsynovial tissues. The loose or areolar areas of the joint surface were penetrated most readily, but the solutions and colloidal dyes also entered the dense areas of the joint surface, and even the cartilage was slowly infiltrated. The material apparently entered the blood stream, because the animal became slowly stained by the dye and only a small part of the dye was found in the regional lymph nodes. A little dye was also taken up by the synovial membrane cells.

A coarse colloid, such as Higgins' American India ink, on the other hand, was removed from the joint with more difficulty. A very small amount of the ink infiltrated the loose areas of the joint surface. Another small portion was taken up by the synovial membrane cells and remained in these cells for many months. The greater part of the ink formed small carbon aggregates which were phagocytized by macrophages (chiefly monocytes) which wandered into the joint cavity and transported the carbon particles through the synovial membrane and into the subsynovial tissues. A fourth method of removal was that some of the ink was caught in masses of fibrin which adhered to the synovial surface. These masses were invaded by the synovial cells which took on the characteristics of fibroblasts and wandered out through the clot to reach its free surface where they formed a new synovial membrane, thus excluding the clot from the joint cavity. Most of the ink remained in the subsynovial tissues for months and was constantly being shifted about by successive generations of macrophages. Some of it was evidently carried back into the joint from time to time by these cells. Some of it lodged in the regional lymph nodes and some of it reached the general circulation and was deposited in the bone marrow. A small part of it was carried by the macrophages into the adjacent bones.

Little is known of the passage of material from the blood stream into the cavities of normal joints. I have noted that dyes such as trypan blue and neutral red, when injected into the ear vein of a rabbit, quickly stain the synovial fluid and the cells in the fluid. In pathological joints, Pemberton has noted that a rise in blood sugar is followed by a rise in the sugar content of the synovial exudate. In joints of patients with syphilis I have found that the Wassermann reaction of the joint fluid was positive when the blood Wassermann was positive, and negative when the blood Wassermann was negative. Somewhat similar findings are reported by Poehlmann (1923).

Boots and Cullen (1922) and Lasch (1926) have studied the hydrogen-ion concentration of the joint exudates in various pathological conditions and found that the P_H varied from 6.14 to 8.4. In chronic conditions the reaction tended to be alkaline and in acute conditions it tended to be neutral or acid. It is probable that the P_H of normal synovial fluid is approximately that of the blood and it is certain that substances can easily reach the joint cavity from the blood stream.

XII. REGENERATION OF THE SYNOVIAL MEMBRANE

This subject has been studied experimentally by Sumita (1912), Segale (1913*a*, *b*), and Key (1925*a*). It has been found that if the synovial membrane is excised under aseptic conditions, it is re-formed by a metaplasia of the connective tissue cells of the subsynovial tissues and that the joint

tends to return to its original condition. In the rabbit this process takes about sixty days.

In the rabbit regeneration took place in the following manner: After the operation there were an effusion of blood into the joint and a leucocytic exudate which lasted about forty-eight hours and then gradually disappeared. The denuded area of the joint surface was then covered by a clot of fibrin and cells. The fixed connective tissue cells in the vicinity enlarged, reverted to the appearance of fibroblasts, proliferated rapidly and invaded the clot. Capillaries also grew in, and by the sixth day the clot was transformed into loose granulation tissue.

As the invading fibroblasts approached the free surface of the clot, they oriented themselves along the surface to become the synovial cells of the new membrane. The fibrin and extravasated blood were removed by macrophages. The cellular proliferation continued for about fifteen days until the layer of granulation tissue was much thicker and more cellular than the normal synovial membrane. After that time it gradually condensed, collagenic fibers were laid down, the fibroblasts disappeared or became mature connective tissue cells, the capillaries decreased in number and the lining of the joint gradually assumed an approximately normal appearance.

XIII. THE SYNOVIAL FLUID

Joints normally contain a small amount of thin glairy fluid which resembles the white of an egg. As a rule, just enough fluid is present to lubricate the joint surfaces. It is alkaline in reaction and contains small amounts of a mucin-like substance, albumin and extractives, fat and inorganic salts.

It has been found that the synovial fluid normally contains from 175 to 225 living cells per cubic millimeter, Key (1925*b*). By means of supravital staining, it was possible to identify these cells as monocytes, clasmatocytes, indeterminate macrophages, polymorphonuclear leucocytes, primitive cells and synovial lining cells. The average of fifty normal synovial fluids from rabbits was as follows: monocytes 58 per cent, clasmatocytes 15 per cent, indeterminate macrophages 14 per cent, leucocytes 5 per cent, primitive cells 1 per cent and synovial lining cells 3 per cent.

The macrophages which comprised about 90 per cent of the cells of most joint fluids examined were mature cells in a state of moderate stimulation and evidently served as scavengers to remove waste material from the joint cavity.

Very few degenerating cells were found in normal joint fluid and very little fat or tissue débris in the fluids of the joints of small animals, but in the fluids of the joints of larger animals fragments of the joint surface

are commonly found. Normally a few red blood cells are present in the synovial fluid and their number is slightly in excess of that of all of the other cells present.

After death, leucocytes in large numbers wander into the joint and the character of the cells in the fluid is considerably changed. In a dog's joint, examined six hours after death, the fluid contained over 2000 cells per cubic millimeter and 60 per cent of these were leucocytes.

In joint exudates, the cells are greatly increased in number. In acute exudates from either trauma or infection the cells are largely leucocytes. In chronic exudates the macrophages usually dominate the picture, and in my experience the monocytes have outnumbered the clasmatocytes regardless of the cause of the exudate.

The origin of the synovial fluid has not been satisfactorily determined. It is not a transudate. I have mentioned the clear vacuoles which are present in many of the synovial cells. It is probable that many of these vacuoles represent a substance which is discharged into the joint cavity, but this has not been proved. Soubbotine (1880) described goblet cells in the synovial surface and regarded the joint lining as a mucous membrane covered by epithelium, but his work is not accepted. In acute tuberculous joints, I have seen tall columnar cells which were apparently shedding their apices or discharging vacuoles into the joint cavity, but I have been unable to stain this material differentially and prove that it is a secretion antecedent which is emptied into the synovial fluid in normal joints. The difficulty is that the process is a very slow one, and it is probable that the mucus of the normal fluid changes little from day to day, although its cellular contents are constantly wandering back and forth through the synovial membrane, and the fluid is in close osmotic relationship with the blood.

XIV. BURSAE AND TENDON SHEATHS

No detailed description will be given of the bursae and tendon sheaths as our knowledge of their structure is very limited. They are cavities similar to joint cavities and contain a small amount of lubricating fluid which resembles the synovial fluid of joints. The walls of bursae and tendon sheaths are lined by a connective tissue membrane which is practically identical with the synovial membrane as described above. This membrane may be of the areolar, collagenic or adipose type, depending upon the character of the tissue upon which it rests; and its cells are modified connective tissue cells which may be on the surface, in the surface or beneath the surface, just as are the synovial lining cells of joints.

XV. BIBLIOGRAPHY

Bentzen, G. E. 1875. Bidrag til ledhulernes udviklingshistorie. *Nordiskt medic Archiv*, **7**, 1; also in Hoffman und Schwalbe, *Jahresbericht*, p. 100.

Boots, R. H., and Cullen, G. E. 1922. The hydrogen ion concentrations of exudates in acute arthritis. *Proc. Soc. Exper. Biol. and Med.*, **19**, 287.

Braun, H. 1894. Untersuchungen über den Bau der synovial Membranen, etc. *Deutsch. Zeitschr. f. Chir.*, **39**, 35.

Cajori, F. A., Crouter, C. Y., and Pemberton, R. 1926. Physiology of synovial fluid. *Arch. Int. Med.*, **37**, 92.

Colomiotti, J. V. 1876. Contribuzione allo studio della articulazione. *Giornale della R. Accademia di Medicina di Torina*, No. 1–2; also in Hoffman and Schwalbe, *Jahresbericht*, p. 119.

Hagen-Torn, O. 1882. Entwicklung und Bau der Synovialmembranen. *Archiv. f. mikr. Anat.*, **21**, 589.

Hammar, A. 1894. Ueber den feineren Bau der Gelenke. *Archiv. f. mikr. Anat.*, **43**, 266, 813.

Heine, J. 1926. Über die Arthritis deformans. *Virchow's Archiv*, **260**, 521.

Hermann and Tourneux. 1880. Contribution à l'étude des membranes synoviales. *Gaz. Méd. de Paris*, **19** (Quoted from Hagen-Torn).

Hueter, C. 1866. Zur Histologie der Gelenkflachen und Gelenkkapseln, mit einem kritischen Vorwart über die Versilberungs Methode. *Virchow's Archiv*, **36**, 25.

—— 1876. Zur Frage über die histologischen Verhaltnisse der Synovalis. *Deut. Zeitschr. f. Chir.*, **6**, 290.

Jaffe, A. 1897. Ueber die Veränderung der Synovialmembran bei Beruhrung mit Blut. *Archiv. f. klin. Chir.*, **54**, 69.

Key, J. A. 1925*a*. The reformation of the synovial membrane in the knees of rabbits after synovectomy. *J. Bone and Joint Surg.*, **7**, 793.

—— 1925*b*. The cytology of the normal synovial fluid. *Anat. Record*, **32**, 235.

—— 1926. The mechanisms involved in the removal of colloidal and particulate carbon from joint cavities. *J. Bone and Joint Surg.*, **8**, 666.

Kroh, F. 1904. Studien über den Bau der Synovialmembran und die Resorption des Gelenkinhalts usw. *Deut. Zeitschr. f. Chir.*, **94**, 215.

Landzert. 1867. Zur Histologie der Synovialhaut. *Centralbl. f. d. med. Wissensch.*, 24.

Lasch, C. H. 1926. Die Bestimmung der Wasserstoffionenkonzentration in Gelenkergussen. *Archiv f. klin. Chir.*, **141**, 129.

Lubosch. 1910. *Bau und Entstehung der Wirbeltiergelenke*. Jena (Quoted from Segale).

Magnus, G. 1923. Über die Resorptionswege aus serosen und synovialen Hohlen. *Deutsch. Zeitschr. f. Chir.*, **182**, 325.

Mosengeil. 1876. Über Massage, deren Technik, Wirkung und Indicationen dazu nebst experimentellen Untersuchungen darüber. *Archiv f. klin. Chir.*, **79**, 428, 551.

Noetzel, W. 1906. Experimentelle Untersuchung über die Infektion und die Bacterien-resorptionen der Gelenke. *Archiv f. klin. Chir.*, **81**, 593.

Poehlmann, A. 1923. Über Gelenksyphilis und serologische Untersuchungen den Gelenkpunkten. *Deut. Zeitschr. f. Chir.*, **182**, 161.

Reyher, C. 1874. On the cartilages and synovial membranes of joints. *J. Anat. and Physiol.*, **8**, 261.

Riedel. 1880. Über das Verhalten von Blut sowie von indifferenten Fremdkorperen in den Gelenken. *Deut. Zeitschr. f. Chir.*, **12**, 447.

Schweigger-Seidel, F. 1867. *Die Behandlung der thierschen Gewebe mit Argent. nitric., etc.* Arbeiten aus der Physiol. Anstalt zu Leipzig, **1**, 150.

Segale, C. 1913*a*. Ueber die Regeneration der synovial Membran und der Gelenkkapsel. Bruns' *Beiträge zur klin. Chir.*, **87**, 259.

—— 1913*b*. Experimentelle Untersuchungen über die Regeneration der Kniegelenkkapsel nach Totalextirpation. *Ibid.*, 299.

van der Sluijs. 1876. Zur Histologie der Synovialhaute. *Nederl. Archiv. f. Zool.*, **3**, 83. (Quoted from Hoffman and Schwalbe, *Jabresbericbt*, 1876.)

Soubbotine, M. 1880. Recherches histologiques sur la structure des membranes synoviales. *Archives de Physiol.*, **7**, 532.

Steinberg. 1874. *Researches on the structure of the synovial membrane.* Inaugural Dissertation. St. Petersburg (Quoted from Soubbotine).

Sumita, M. 1912. Experimentelle Beiträge zur operationeller Mobilisierung ankylosierter Gelenke. *Arcbiv f. klin. Cbir.*, **98**, 755.

Tillmans, H. 1874. Beiträge zur Histologie der Gelenke. *Arcb. f. mikr. Anat.*, **10**, 401.

—— 1875. Zur Histologie der Synovialmembranen. *Arcb. f. klin. Cbir.*, **19**, 693.

——— 1876. Untersuchungen über die Unzuverlassigkeit der Versilberungs Methods für die Histologie der Gelenke. *Virchow's Arcbiv*, **67**, 398.

——— 1876. Die Lymphgefasse der Gelenke. *Arcb. f. mikr. Anat.*, **12**, 649.

Weichselbaum. 1877. Die senilen Veränderungen der Gelenke und deren Zusammenhang mit der Arthritis deformans. *Sitzungsbericht d. k. Akad d. Wissenchaft*, Wien, III Abt., **75**, 193.

PLATE I

FIG. 318.—Areolar type of synovial membrane from the lateral capsule of the knee joint of a rabbit. Small villi in cross and long section at the right. 8 mm. objective and ocular 8.

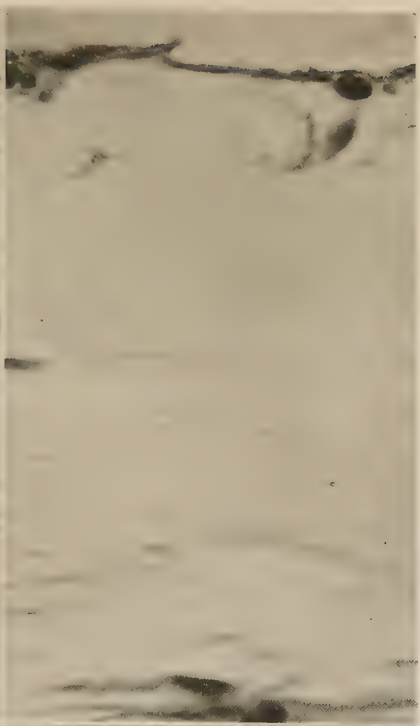
FIG. 319.—Areolar type of synovial membrane from the roof of the quadriceps pouch of the knee joint of a rabbit. A small vessel very near the surface is seen at the right. 2 mm. objective and ocular 8.

FIG. 320.—Areolar type of synovial membrane from the lateral capsule of the knee joint of a dog. An unusually cellular and vascular area. 2 mm. objective and ocular 8.

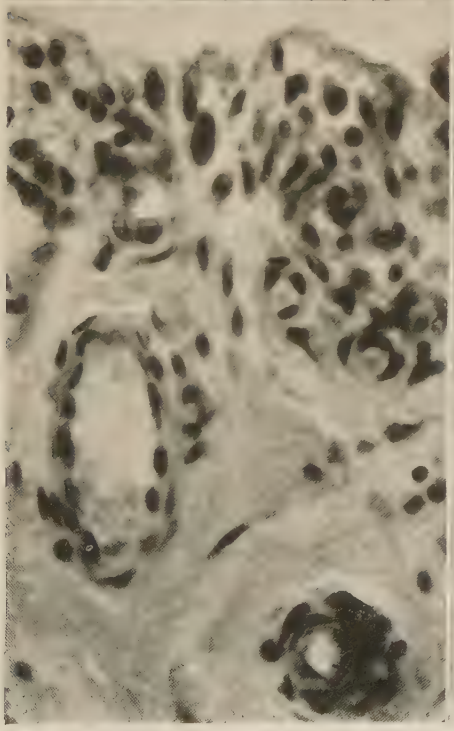
FIG. 321.—Areolar type of synovial membrane showing folds or villi, from the lateral capsule of the knee joint of a man fifty-one years of age. 8 mm. objective and ocular 8.

PLATE I

318



319



320

321

PLATE II

FIG. 322.—Smooth area from the lateral capsule of the same joint as the preceding, areolar type. 8 mm. objective and ocular 8.

FIG. 323.—Transition stage between the areolar and fibrous type of synovial membrane from the side of the femur in the knee joint of a dog. 4 mm. objective and ocular 8.

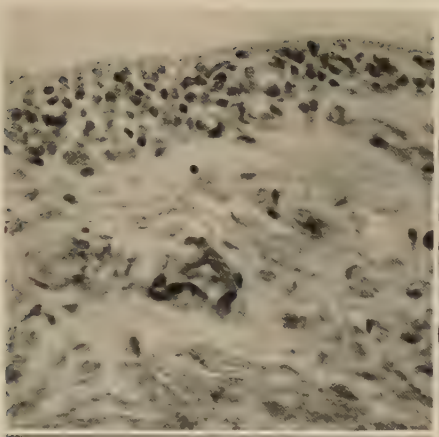
FIG. 324.—Fibrous type of synovial membrane from the lateral capsule of the same joint as Figure 321. Note the location of the cells beneath the surface. 4 mm. objective and ocular 8.

FIG. 325.—Fibrous type of synovial membrane from the side of the femur in the same joint as Figure 321. Note the tendency of the cells to lie in fibrous lacunae. 4 mm. objective and ocular 8.

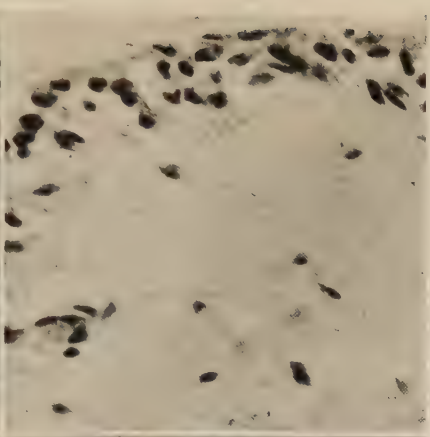
FIG. 326.—Band of fibrous synovial membrane surrounded by the areolar type in the lateral capsule of the knee of a rabbit. Note the small folds or villi on either side. 16 mm. objective and ocular 8.

FIG. 327. Adipose type of synovial membrane from the knee joint of a woman aged seventy years. 8 mm. objective and ocular 8.

PLATE II

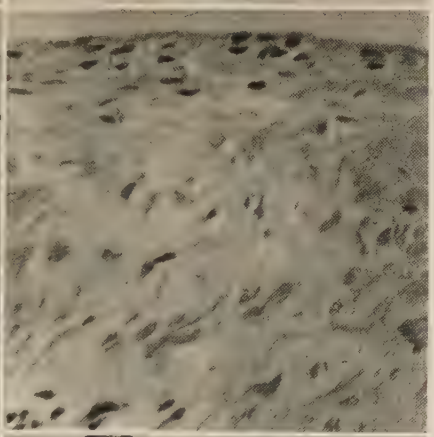


322



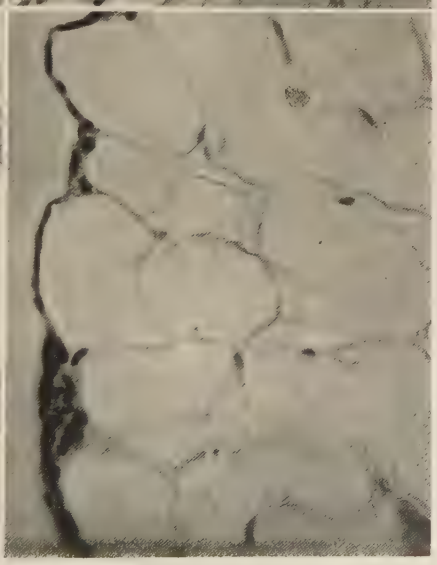
323

324



325

326



327

PLATE III

FIG. 328. Three drawings from Hammar probably from human joints: *a*, from the hip and *b* and *c* from shoulder joints. Note the cell processes. In *c* the surface cells marked *z*, are encapsulated, and the deeper cells marked *z* are not.

PLATE III

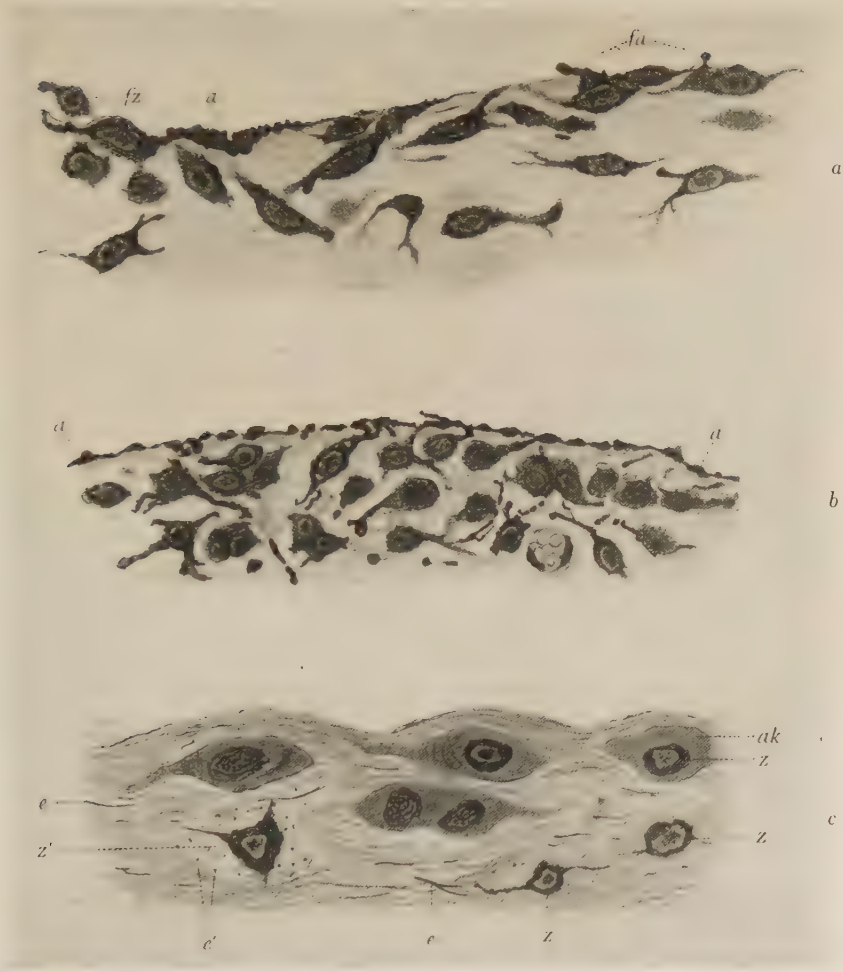


PLATE IV

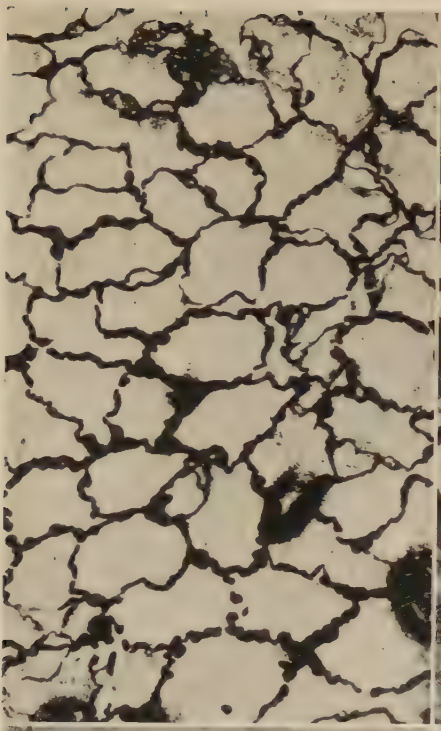
Fig. 329.—Silver nitrate preparation from the lateral capsule of the knee joint of a rabbit. 4 mm. objective and ocular 8.

Fig. 330.—Similar preparation from the border of the quadriceps tendon of the same joint. 4 mm. objective and ocular 8.

Fig. 331.—Border of the semilunar cartilage from the knee joint of a rabbit showing the marginal fold and the transition zone of the synovial membrane.

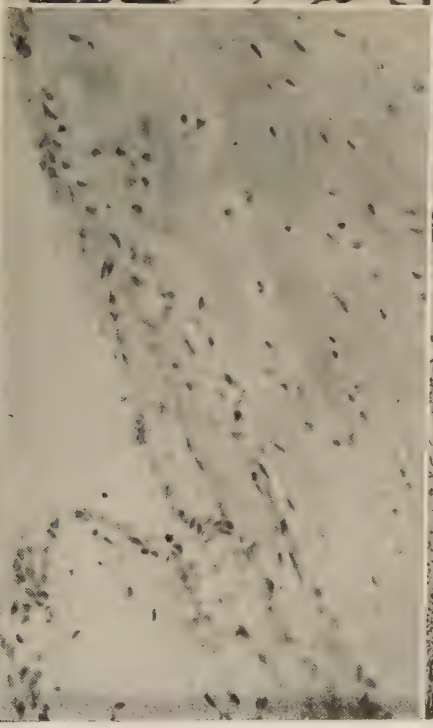
Fig. 332.—Large villus from the same joint as Figure 321. Note the numerous blood vessels and the small secondary villi. 16 mm. objective and ocular 8.

PLATE IV

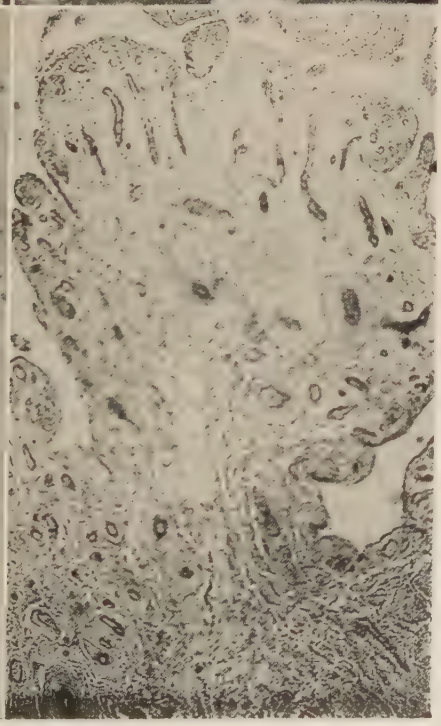


329

330



331



332

PLATE V

FIG. 333. Drawings from Hammar. Free surface of prepatellar bursae; villus projecting from synovial membrane.

PLATE VI

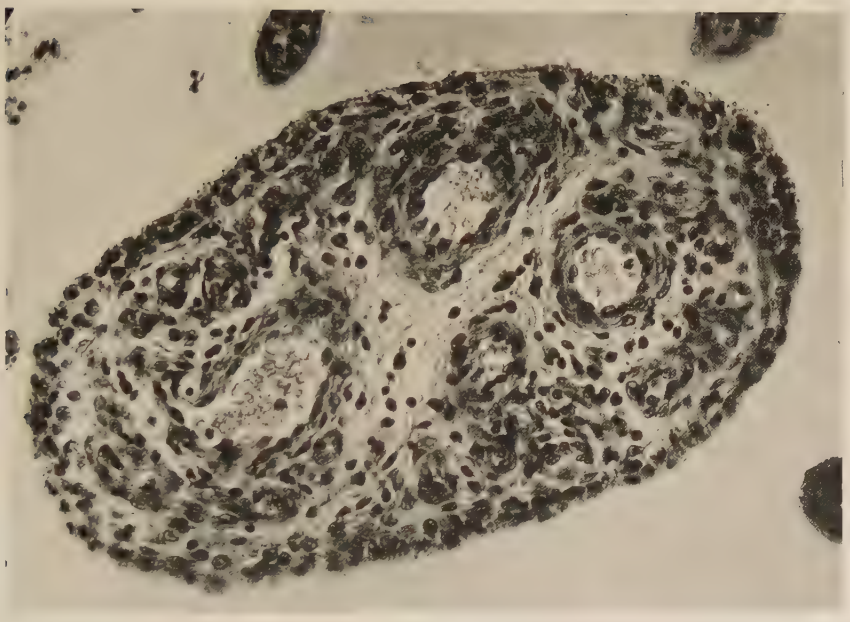


Fig. 334.—Cross section of a vascular villus from the same joint as Figure 321. 4 mm. objective and ocular 8.

Section XXII

STRIATED AND SMOOTH MUSCLE

CONTENTS

Section XXII

PAGE

I. Striated Muscle 769
1. Appearance of surviving vertebrate muscle fiber 769
2. Appearance of uncontracted insect's wing muscle 771
3. Comparison between insect's wing muscle and vertebrate muscle in uncontracted state 774
4. Changes undergone by striated muscle during contraction 777
5. Brief discussion of views of various investigators in relation to that above set forth 781
6. Portions of structure of striated muscle which are not connected with contraction 783
7. Pathology of striated muscle 783
8. Osmotic properties of striated muscle in relation to its function 784
9. Relation between structure of striated muscle and biochemistry of contraction 787
II. Smooth Muscle 789
1. Structure of vertebrate smooth muscle 789
2. Changes undergone by smooth muscle fibers during contraction 792
3. Portions of structure of smooth muscle which are not directly connected with contraction 795
4. Osmotic properties of smooth muscle in relation to its function 795
5. Biochemistry of contraction in smooth muscle 798
III. Concluding Remarks 799
Details regarding illustrations 801
IV. Bibliography 802

Section XXII

STRIATED AND SMOOTH MUSCLE

EDWARD B. MEIGS

FOR the last hundred years, human beings have been occupied with the construction of machines, by means of which chemical energy can be applied to the movement of large bodies against such forces as friction and gravitation. During the same period, the mechanisms contained in animals and plants have been intensively studied, and it has been found that these mechanisms present certain similarities to the machines of human invention. In particular, the muscles by which animals move themselves about have long been known to carry on their activities with an accompaniment of the oxidation of carbon and hydrogen compounds in some respects analogous to that which occurs in steam and gasoline engines, and a long line of investigators have devoted themselves, on the one hand, to the study of the chemical changes which take place in active muscle, and, on the other, to the visible structure by means of which these chemical activities are applied to the forcible movement of large masses of matter.

Muscle may, therefore, be regarded as a kind of machinery, by means of which chemical energy is applied to producing the forcible tension characteristic of contraction, and the study of its structure from this point of view demands a somewhat different outlook and somewhat different methods from that of tissues whose activities are purely chemical, and not connected with visible change of form and movement. The whole muscle is capable of becoming forcibly shorter and thicker, and it has been established that this shortening and thickening of the whole muscle is the result of the shortening and thickening of each of its fibers. Is there anything in the visible structure of the muscle fiber and in the visible changes which it undergoes during contraction to suggest how it carries out the conversion of chemical energy into mass motion?

I. STRIATED MUSCLE

1. *The appearance of the surviving vertebrate muscle fiber:*

Striated muscle has a very characteristic appearance in all the vertebrate animals. When placed in solutions which contain the same salts as the blood plasma in about the same quantities, it retains its power of contracting in response to an electrical current for some time after it has been removed from the body. When teased with needles on a glass slide in such solutions, it readily separates into its individual fibers. Each of these fibers evidently represents a union of several cells, for each contains a number of nuclei. The diameter of the fibers and the arrangement of the nuclei differ

somewhat in different species of vertebrates, but in all vertebrates the fibers show a characteristic and striking cross striation. The alternating lighter and darker transverse lines which make up the cross striation are at approximately equal intervals in most vertebrates. In addition to the transverse lines, careful study reveals that less conspicuous lighter and darker lines run at right angles to these along the length of the fiber.

Figure 335 is the reproduction of a photograph of vertebrate muscle fibers. The characteristic transverse and longitudinal striations are well shown, and the appearance of the nuclei is indicated. The appearance of

FIG. 335.—Photograph of striated mammalian muscle, showing the usual appearance under moderately high magnification. × 700.

striated muscle in vertebrates is so constant that that shown in Figure 335 may be taken as representing fairly well the fibers of any vertebrate.

The study of this figure shows that the muscle fiber is made up of at least two different substances with different refractive indices and different tendencies to absorb transmitted light, which are arranged so that the surfaces of separation form fairly complicated figures. It is obvious from purely optical considerations that the forms of these surfaces of separation cannot be accurately determined from a microscopic study of the whole fibers, however careful. The appearance of that part of the fiber which is immediately in focus is so much distorted by the parts lying above or below the plane of focus that it is impossible to make out the details with any cer-

tainty. Still less is it possible to make out from the appearance of the whole fiber the physical and mechanical peculiarities of the parts which make it up.

It has never been possible to separate the fibers of vertebrate muscle into smaller elements without subjecting them to the action of reagents which completely destroy their function. But studies of the wing muscle of insects have thrown a flood of light on the whole subject of the microscopic anatomy and physiology of muscle, and the results of such studies will now be considered.

2. *The appearance of uncontracted insect's wing muscle:*

When the wing muscle is dissected out from the thorax of a fly and teased apart in 0.7 per cent sodium chloride solution or in white of egg, it readily breaks up into fibrils which are much smaller than the fibers of vertebrate muscle, and evidently simpler in their structure. These fibrils have been described by a number of histologists (Merkel, 1872; Schäfer, 1891; Janet, 1907) and photographs of them have been published by McDougall (1897) and the writer (1908*a*). Figure 336, reproduced from the last-mentioned article, shows a surviving wing fibril of the fly (*Calliphora Vomitoria*), as it appears when photographed by ultraviolet light. Photographs taken by ultraviolet light can be advantageously magnified somewhat more than when ordinary light is used. Further details regarding this and the other illustrations are given in a paragraph at the end of this chapter and in the legends accompanying the illustrations.

The fibril shown in Figure 336 is magnified 1800 diameters, and, as can readily be estimated from this, it has a diameter of about 2.2μ, and is crossed at intervals of 3μ by well-marked transverse lines. Midway between two of these well-marked lines, a much fainter line can often be made out.

It will be noted that, in the fibril shown in Figure 336, the transverse lines appear dark at the lower end and bright at the upper end. This is due to the fact that the fibril lay somewhat oblique to the plane of focus when it was photographed. By careful microscopic study of the fresh fibrils it can easily be made out that the transverse lines appear dark when they are a little above the plane of focus; and bright, when they are below.

On one side of the dark transverse lines in the lower part of the fibril, a narrow bright line sometimes appears, but the appearance of this bright line is quite irregular. It varies greatly in breadth and brightness, and is probably a purely optical effect rather than the expression of another substance in the fibril.

The above-mentioned features of the cross striation of voluntary muscle have been described by a great number of investigators, but the descriptions of the cross striation are generally much more complicated than that which has just been given. For the present, the significance of the additional features of the cross striation will not be discussed; those mentioned above

will be designated so that they can be identified with the corresponding features in other descriptions according to the most commonly accepted usage. The well-marked dark transverse lines as they appear at the lower end of the fibril will be called z; the bright line, which sometimes appears on one side of z, j. The broad dim space which occupies by far the greater proportion of the volume of the fibril will be called q; the faint transverse line, midway between two z's m; and the faintly lit-up space which sometimes appears in the neighborhood of m, h. The division of the fibril included between two neighboring z lines will be spoken of as a sarcomere. The manner in which the various cross striations are to be lettered is shown in Figure 336.

The fibrils of insects' wing muscle have been called sarcostyles by previous authors (Schafer, 1891, p. 203), and this term will be used in the following discussion of them.

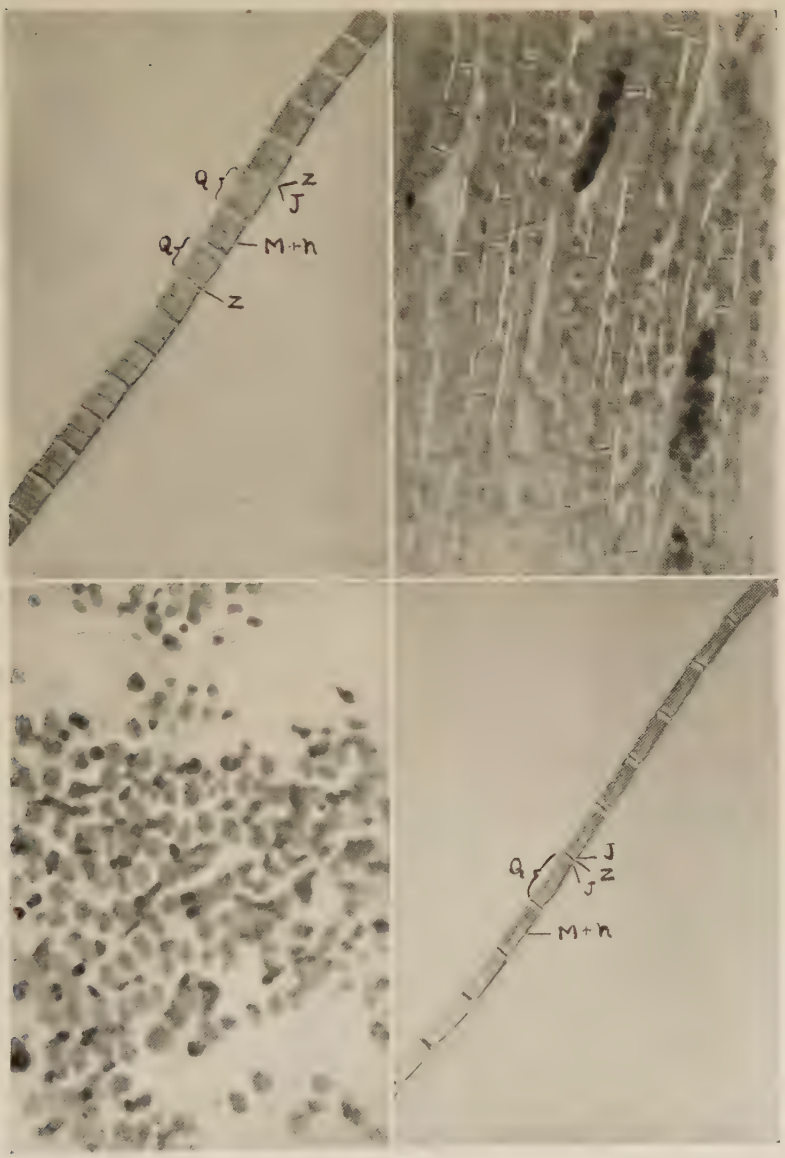
The cross striation of the insects' sarcostyles is different in many respects from that which appears in the fibers of vertebrate muscle. But it may at least be suspected that the machinery of contraction is fundamentally similar in the two cases. The following line of reasoning and research has been pursued by the writer in order to determine the similarities and differences between the two tissues.

Insects' sarcostyles teased out in the fresh state have been observed to contract beneath the microscope (Rollett, 1885; Schafer, 1891, p. 203; McDougall, 1897, p. 413), and it may therefore be considered that their appearance is that of the living tissue. It is easy, of course, to subject them to any desired histological process, and thus to determine what changes are produced in their appearance by the procedure in question.

The appearance of the living vertebrate muscle fibers suggests that they are composed of smaller fibrils. It has been impossible to break up the living fibers into smaller fibrils, but this can be done after they are treated with alcohol. It is possible, therefore, to compare the isolated fibrils of vertebrate muscle after histological treatment with the insects' sarcostyles after similar treatment, and this procedure has been carried out by the writer.

Small pieces of wing muscle have been dissected out from flies which were drowned in 70 per cent alcohol, and have been embedded in paraffin and cut into sections about 2μ thick. These sections have been preserved in glycerin on quartz slides and photographed by ultraviolet light. Figure 337 is a reproduction of such a photograph.

If the sarcostyles, as they appear in Figure 337 after the histological treatment, are compared with the fresh sarcostyle shown in Figure 336, the following differences will be noted. There is a considerable lateral shrinkage, the fixed sarcostyles having a diameter of about 1.3μ as compared to 2.2μ in the fresh sarcostyle. The sarcomeres of the former are somewhat longer, however, than those of the latter. This is not surprising, in view of the fact



336 337

338 339

FIG. 336.—Surviving sarcostyle of fly's wing muscle. 0.7 per cent NaCl × 1800.

FIG. 337.—Longitudinal section 2½μ thick of fly's wing muscle, showing nuclei. Alcohol fixation; paraffin; glycerin. × 1800.

FIG. 338.—Cross section 1¼μ thick of fly's wing muscle. Alcohol fixation; paraffin; glycerin. × 1800.

FIG. 339.—Isolated sarcostyle from fly's wing muscle. Alcohol fixation; glycerin. × 1800.

that the former were hardened in situ in the fly's thorax, while the latter are lying free in 0.7 per cent salt solution and subject to no extending force.

The appearance in the neighborhood of z has been altered by the histological treatment; z now appears as a rather fine dark line bordered with some regularity by narrow bright lines. These bright transverse lines beside z will be called j as in the surviving sarcostyle. It seems likely that the histological treatment has produced this change in appearance by altering the relation between the indices of refraction of the substances composing z and q, but a full discussion of this question will be reserved until later. The appearance of m and h is not so regular or so marked in the fixed tissue as in the fresh, but a careful examination shows that this part of the cross striation also can sometimes be seen. On the whole it may be said that many of the features of the appearance of the fresh tissue reappear in the histological preparations. Figure 338 is a cross section of fly's wing muscle.

The spaces between the sarcostyles are, of course, filled with fluid in the living muscle, and this fluid has been called sarcoplasm by previous investigators. The word sarcoplasm will be used to designate this fluid in the subsequent discussion and the spaces which appear between the sarcostyles in histological preparations will be called the sarcoplasmic spaces.

Scattered through the sarcoplasmic spaces in the sections which have just been described are small dark granules. Granules appearing in the sarcoplasm of insects' wing muscles have been described by several investigators (Schafer, 1891; Meyer, 1921, Part II, p. 731), but their significance is still very doubtful.

Figure 339 is an insect's sarcostyle which has been hardened in alcohol and teased out in glycerin. The various features of the cross striation have been lettered in this figure.

3. *Comparison between insect's wing muscle and vertebrate muscle in the uncontracted state:*

The evidence that all vertebrate striated muscle has an essentially similar structure is so strong that there need be no hesitation in using frog's muscle as a type for the tissue.This procedure has the advantage that frog's muscle is easily obtained, and lends itself readily to physiological study, so that in it the results of histological and physiological research can be satisfactorily coordinated. Frog's muscle can be fixed in 70 per cent alcohol without undergoing much shortening, and it is to be presumed that preparations from tissue fixed in this way represent uncontracted muscle. Tissue fixed in this way can be embedded in paraffin and cut into very thin longitudinal or transverse sections.

Figures 340 and 341 are reproductions of photographs by ultraviolet light of an unstained longitudinal section of frog's striated muscle, prepared

in every way like the section of insect's muscle represented in Figure 337. A comparison of the two kinds of muscle as they appear in these preparations justifies the statement that the frog's muscle is made up of sarcostyles which are essentially similar to those of the insect's wing muscle. The cross striation has very much the same character in the two kinds of tissue, and is subject to the same variations in appearance. At a, a, Figure 340, the striation Q + J + Z + J + Q can be fairly well seen. In these portions of the field the sarcostyles lie somewhat above the plane of focus, the Z lines appear dark, and the J lines appear brighter than either Z or Q. At b in Figure 341, on the other hand, one of the sarcostyles lies below the plane of focus, the Z lines appear brighter than any other part of the sarcostyle and the J lines appear darker than either Z or Q. In the diagonal sarcostyle shown at b, also the M lines appear fairly plainly as bright lines midway between each pair of neighboring Z lines.

The frog's sarcostyles are a good deal smaller than those of the insect's wing muscle, the sarcomeres having a diameter of about 1μ and a height of about 1.7μ as compared to 1.3μ and 4μ respectively in the fixed insect muscle. The relation between the height and diameter of the fixed frog's sarcomeres is more like that of the fresh than of the fixed insect's sarcomeres. It seems not unlikely that the frog's sarcostyles may have less tendency than those of the insects to shrink in the lateral direction when fixed.

An interesting feature of the frog's muscle is to be seen in the regions marked c, c, c in Figure 340. In these regions dark lines may often be seen extending laterally from the ends of Z, and sometimes of M also, into the spaces surrounding the sarcostyles. Perhaps the most noticeable differences between vertebrate muscle and fly's wing muscle are that, in the former, large bundles of sarcostyles are bound together into fibers which cannot be teased apart while they are still living, and that the cross striations of all the sarcostyles in each fiber of vertebrate muscle are held at the same levels, so that the fibers show their characteristic cross striation. It seems likely that the dark lines shown at c, c, c in Figure 340 are the expression of a system of transverse membranes, which, in the living state, bind the sarcostyles into a muscle fiber and hold the cross striations of all the sarcostyles composing a fiber at the same levels. That the fibrils or sarcostyles within the cross-striated muscle fiber are held together by a system of cross connections through the sarcoplasm is in harmony with the views of earlier and later investigators (Heidenhain, 1898, pp. 50 et seq.; Bailey, 1925, p. 152).

The lateral outlines of the sarcostyles often bulge out somewhat between the Z lines in the frog's muscle, so that each sarcomere appears barrel-shaped instead of perfectly cylindrical. This appearance is quite plainly shown at b in Figure 341.

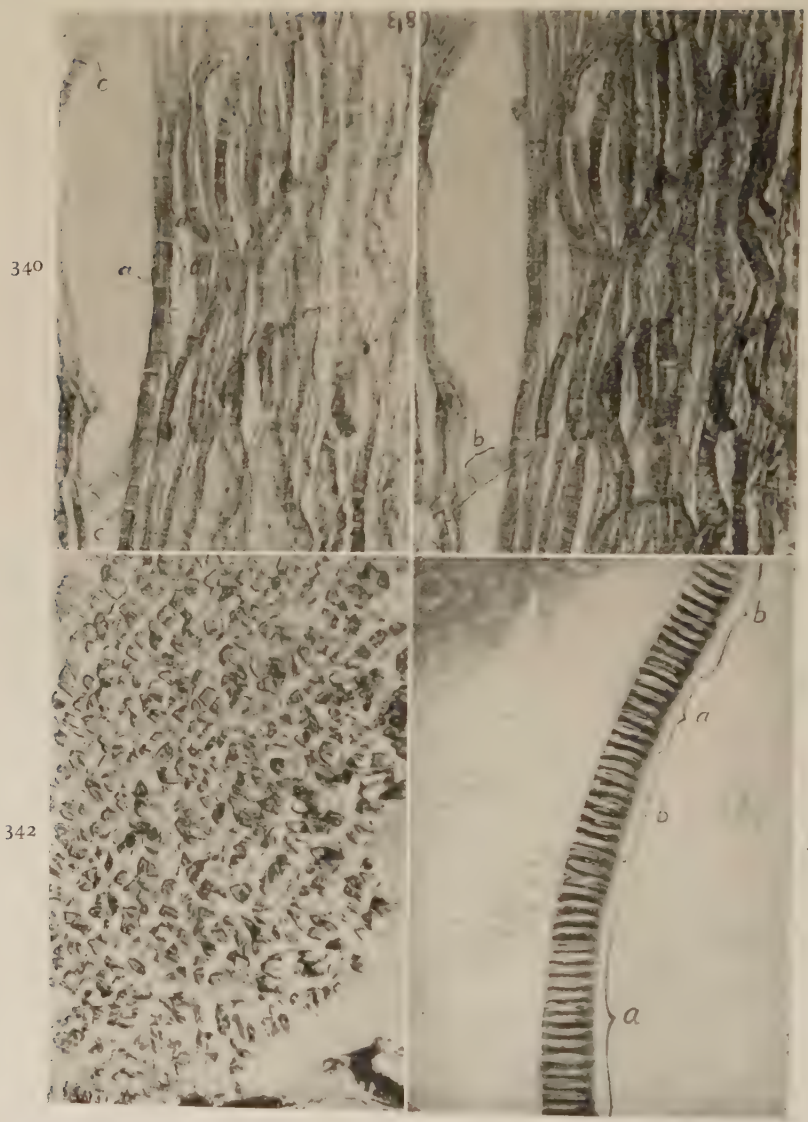


Fig. 340.—Longitudinal section $1\frac{1}{4}\mu$ thick of uncontracted frog's muscle. Alcohol fixation; paraffin; glycerin. × 1800. a, a, regions in which appearance of Q + J + Z + J + Q, etc., is fairly well seen; at c, c, c are seen remains of membranes, which, in the living state, bind the sarcostyles together.

Fig. 341.—Photograph at slightly different focus of the same field as that of Fig. 340. At the place marked b, the M and h lines are somewhat better seen than in Fig. 340.

Fig. 342.—Cross section $1\frac{1}{4}\mu$ thick of uncontracted frog's muscle. Alcohol fixation; paraffin; glycerin. × 1800.

Fig. 343.—A sarcostyle from fly's wing muscle teased out fresh in a mixture of equal parts white of egg and 2 per cent NaCl solution. × 1300.

Figure 342 shows a cross section of frog's striated muscle. This preparation indicates that the frog's sarcostyles are somewhat more irregular in shape and size than are those of the insect wing muscle.

4. *The changes undergone by striated muscle during contraction:*

There are great difficulties in the way of determining even the main features of the visible changes undergone by the muscle sarcostyles during contraction. One of these difficulties is the small size of the sarcostyles, and another is connected with the physiological peculiarities of contraction in striated muscle. Not only is it very rapid, but it has also the peculiarity that it is not maintained under normal conditions except by a rapid series of stimuli and a rapid output of chemical energy. It is questionable, therefore, whether it is possible to fix striated muscle by histological agents in a state of normal contraction. It seems quite probable that histological reagents applied to muscle in this state would interfere with the active chemical processes which constitute it before there is time for the muscle to be fixed.

Striated muscle can be thrown into a state of maintained contraction by certain chemical reagents and by heat, and the writer believes that light is to be obtained on the nature of contraction by studying it histologically after it has been thrown into this state. But there are complications also about this way of approaching the subject. Many tissues, among which connective tissue is conspicuous, shorten markedly when heated to about 55°C., and muscle heated to that temperature shortens much more than it does under physiological conditions. It seems likely that the shortening of connective tissue and a part of the shortening of muscle under these conditions is of an entirely different nature from normal contraction.

For all these reasons, it is not surprising that there is no general agreement as yet in regard to the visible changes undergone by the muscle sarcostyles during normal contraction. The writer holds a certain view which seems to him the most likely interpretation of the facts as they stand at present, and which will be presented in the following pages. This view is in conflict with those of certain other observers, and it is not possible in a short treatment of the subject to give any satisfactory account of the reasons for and against the various views that are held. But the attempt will be made to keep observation and interpretation separate as far as possible, and the references given will enable the reader to follow any of the other views of the subject, which may appear probable.

Investigators who have studied the sarcostyles of insects' wing muscle have described their appearing in a form in which they are bulged out in the intervals between the z lines so that they assume the form of a string of beads. Merkel (1872, Fig. 8) gives a picture of such a beaded sarcostyle, and states that it had been treated with acetic acid. Ranvier (1880) regards the beaded form as characteristic of contracted sarcostyles. Schafer (1891,

Fig. 25) also gives a picture of such a sarcostyle, and states that it is contracted. McDougall (1897,1898) bases his theory of contraction largely on the view that the beaded form is characteristic of contracted sarcostyles.

When the sarcostyles of fly's wing muscle are teased out fresh in a mixture of white of egg and 2 per cent sodium chloride solution, they often have the appearance shown in Figures 343, 344 and 345. The magnification in these three figures is only 1300 diameters.

If the sarcostyles shown in Figures 343 to 345 are compared with the uncontracted sarcostyle shown in Figure 336, it will be seen that the former have a considerably greater diameter and that their dark transverse lines are much closer together. The sarcomeres of the uncontracted sarcostyles have a diameter of about 2.2μ and a height of 3μ; while those of the sarcostyles shown in Figures 343 to 345 have a diameter of from 4.5μ to 6μ and a height of from 1.3μ to 0.8μ. The sarcostyles shown in Figures 343 to 345 have therefore shortened and increased in diameter, and may be regarded, at least provisionally, as contracted sarcostyles.

If attention be directed to the edges of the sarcostyles at the places marked a in Figures 343 and 344, it will be seen that they are not straight lines, but form a series of curves. The appearance strongly suggests that when a sarcostyle contracts, it bulges out in the intervals between the z lines, so that these latter form a series of deep constrictions all along the course of the contracted sarcostyle.

In many places in Figures 343 to 345 transverse lines can be seen crossing the sarcostyles midway between the two z lines in the positions corresponding to those of the M lines in the uncontracted sarcostyles.

The writer has endeavored to determine whether the contracted sarcostyles of vertebrate muscle have a beaded form similar to that which has been observed in the insect's wing muscle. Pieces of frog's muscle have been immersed in 70 per cent alcohol while under the influence of a rapidly interrupted faradic current, and have later been embedded in paraffin and cut into thin sections. In such preparations, however, many of the sarcostyles appear little different from those of uncontracted muscle.

Other pieces of muscle have been fixed in 70 per cent alcohol after they had been caused to go into a state of permanent shortening by heating them to from 45° to 50°C. This is, of course, an abnormal form of shortening. But there is reason to believe that it is similar in some respects to the normal contraction. It is accompanied by lactic acid formation, which occurs also in normal contraction; it has about the same extent as the extreme normal contraction; and it is characteristic of muscle in the sense that other tissues such as tendon do not shorten when heated to between 45° and 50°C.

Pieces of muscle fixed in heat contraction as above described have been embedded in paraffin and cut into longitudinal sections 2½μ thick. Figure 346 is the reproduction of a photograph by ultraviolet of such a section

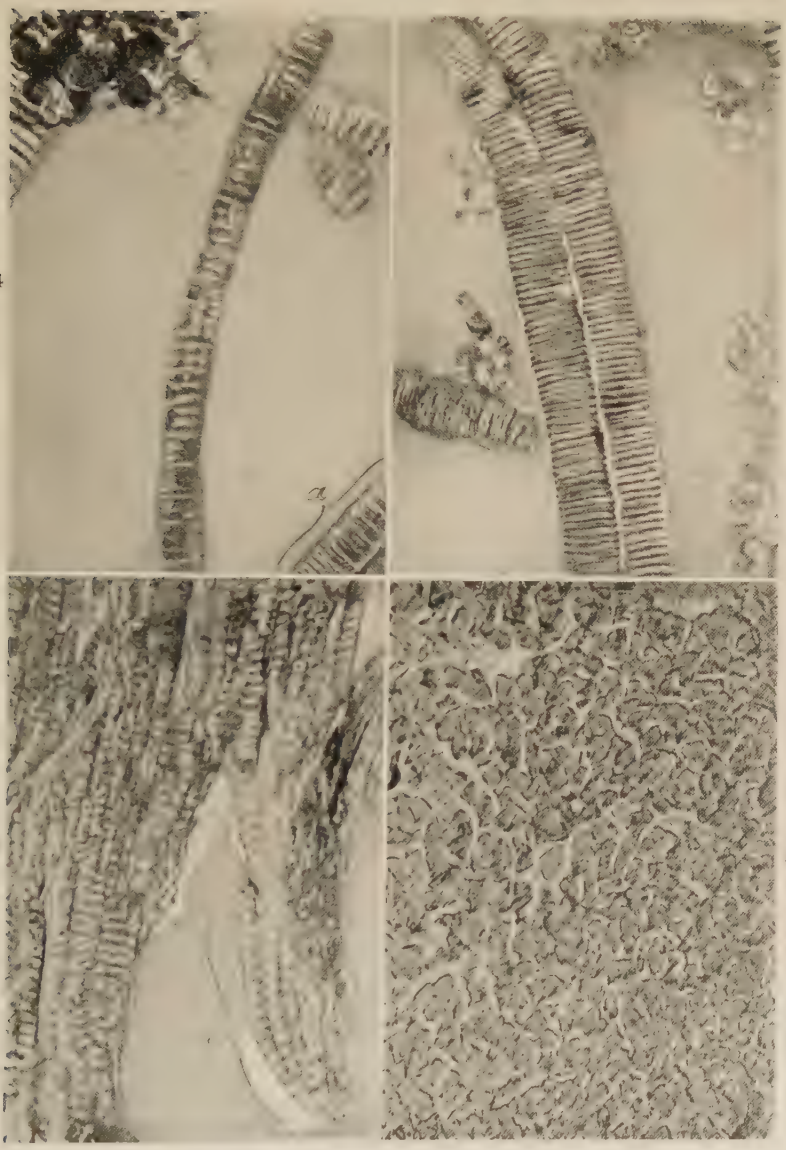


FIG. 344.—Sarcostyles from fly's wing muscle teased out fresh in a mixture of equal parts white of egg and 2 per cent NaCl solution. × 1300.

FIG. 345.—Sarcostyles from fly's wing muscle teased out fresh in a mixture of egg and 2 per cent NaCl solution. × 1300. The degree of contraction is extreme.

FIG. 346.—Longitudinal section 2½μ thick of frog's striated muscle in heat rigor. Alcohol fixation; paraffin; glycerin. × 1800. At a the beaded form of one of the sarcostyles is well shown.

FIG. 347.—Cross section 1¼μ thick of frog's striated muscle in heat rigor. Alcohol fixation; paraffin; glycerin. × 1800.

magnified 1800 diameters. The distance between neighboring z lines in this photograph is about half that in the photograph of the uncontracted muscle shown in Figure 340.

If the sarcostyles of the frog's muscle shown in Figure 346 are compared with those of the insect's muscle shown in Figure 343, it will be seen that there are many points of similarity. The z lines have the same heavy character in both cases, and there is the same evidence that in both cases the sarcostyles show a series of bulgings between each neighboring pair of z lines. The evidence suggests that the two kinds of sarcostyles contract by bulging out in the intervals beween the z lines while these latter are drawn nearer together.

The remarkable experiments of Berry Haycraft show that the sarcostyles of striated muscle commonly have a beaded form, and that the appearances which have been interpreted in the foregoing paragraphs as indicating that they have this form are not an optical delusion. Haycraft has pressed pieces of fresh muscle against layers of soft collodion, with the result that the collodion afterwards shows a longitudinal and transverse striation corresponding to that of the muscle (Haycraft, 1891). Professor W. Biedermann of the University of Jena has successfully repeated this experiment.*

The beading of the sarcostyles during contraction suggests that the substance of the z lines or discs is less extensible than other parts of the structure, and that there is a tendency for the sarcostyle to be distended during contraction which causes it to bulge out in the intervals between these discs. The distending force which causes this change of form might be the absorption by the sarcostyle of some of the sarcoplasmic fluid by which it is surrounded, in which case the contracted sarcostyles would have a greater volume than the uncontracted ones.

McDougall has made measurements from which he has calculated the volume of the sarcomeres of insects' wing muscle in various stages of contraction (1897, pp. 422 and 423). It seems from his description that these measurements were made by direct microscopic observation (not from photographs), and he does not consider them accurate enough to decide the question whether or not the sarcostyles increase in volume during contraction. As far as they go, they seem to indicate that if any increase in volume does take place, it is not more than 30 per cent of the volume of the uncontracted sarcomeres.

It is much easier to measure the sarcostyles in such photographs as those reproduced in Figures 336, 343 to 345 than to measure them as they appear directly under the microscope. The uncontracted sarcomeres are nearly cylindrical in form, and their volume may be calculated by multiplying the height by π times the square of the radius. They have a volume of about 11

* Personal communication.

cubic μ. In the case of the contracted sarcomeres, the volume can only be estimated on account of the bulgings between the z lines. The diameter at the levels of the constrictions caused by the z lines may, however, be measured, and the volume of a cylinder with this diameter and the height of the sarcomere may be calculated. These cylinders in the contracted muscle have a volume of about 1⁻ cubic μ, that is, about 50 per cent more than that of the uncontracted sarcomeres. The contracted sarcomeres themselves, of course, have a somewhat greater volume.

Measurements of the contracted and uncontracted frog's sarcostyles are less satisfactory than those of the insects' sarcostyles on account of their variation in size. But a comparison of cross sections of contracted and uncontracted frog's muscle points to the same conclusion as do the measurements of the contracted and uncontracted insects' sarcostyles. A cross section of frog's muscle in heart rigor is shown in Figure 347 and is to be compared with the cross section of uncontracted muscle shown in Figure 342. It is very obvious that the relation between the areas occupied by the sarcostyles and by the interstitial spaces is different in the two preparations. In the contracted muscle the interstitial spaces are much smaller in relation to the sarcostyles, which indicates that during contraction fluid has passed from the former to the latter.

The question whether the sarcostyles of striated muscle increase in volume during contraction has been touched on by Eycleshymer, Hürthle, Gutherz and Marcus. Eycleshymer (1904, p. 293), Hürthle (1907, pp. 122 to 124), and Gutherz (1910) have observed that in cross sections of contracted muscle the sarcostyles appear more closely packed together than in similar sections of uncontracted muscle, which would indicate that fluid passes from the sarcoplasmic spaces to the sarcostyles during contraction. Later, however, Hürthle (1909) came to the conclusion that his measurements of relaxed and contracted sarcostyles did not indicate any change in volume during contraction. The evidence for this view did not seem strong to the writer and his reasons for doubting it were published (Meigs, 1914). Hürthle replied to some of these reasons (1914), but admitted that his measurements were not sufficient finally to decide the question. More recently Marcus (1919 to 1921) has made measurements of the relaxed and contracted sarcostyles of insect's muscle, which lead him to believe that there is no change in volume during contraction, though he expresses himself cautiously.

5. Brief discussion of the views of various investigators in relation to that above set forth:

The histological study of striated muscle shows that it is made up of minute threads along which two sets of transverse membranes occur at regular intervals. In the wing muscle of most insects the threads are exceed-

ingly uniform in size and shape, and the membranes occur at very uniform intervals. In vertebrate muscle, as it appears in histological preparations, the diameter of the threads is somewhat more variable than in the insect muscle, but here also the intervals at which the transverse membranes occur are exceedingly uniform. There is a good deal of evidence to indicate that during contraction the threads absorb fluid from the spaces between them and become bulged out in the intervals between the transverse membranes.

The preceding paragraph seems to the writer a fair outline of the most important points in our present knowledge of the histology of striated muscle and of the microscopic changes which it undergoes during contraction. Before leaving this part of the subject, however, it is necessary to point out that it is almost impossible to make any statement which will not be in conflict with some of the published descriptions of uncontracted muscle, and that the accounts of the changes which occur during contraction also contain a good many direct or implied contradictions.

Rollett (1885, 1886) and many other histologists (Schafer, 1920, p. 133; Bailey, 1925, p. 151) believe that those portions of the sarcostyles which lie between the Z and M membranes are made up of several transverse discs of different substances with different staining reactions and different appearances in polarized light. The fibrils or sarcostyles have often been pictured with a quite different series of bulgings and constrictions from those described for contracted muscle in the foregoing pages (Heidenhain, 1898, p. 90). Some authors believe that the sarcostyles are surrounded by membranes (McDougall, 1897; Marcus, 1919 to 1921); Heindenhain (1898, p. 69), and Janisch (1923 to 1924), on the other hand, think that they are made up of still finer fibrils; while Schafer (1920, p. 133) thinks that they contain a complicated system of tubules.

Many of these descriptions are difficult to reconcile with each other or with what can be seen in surviving muscle. The writer has made a study of the appearances which are produced in muscle by certain histological procedures and by slight differences of focus (Meigs, 1908*a*); it seems likely that a good deal of the confusion which exists at present in the literature will ultimately be explained as due to the great optical difficulties in the way of studying such minute objects as the muscle sarcostyles and to the rather unpredictable effects of histological treatment (Biedermann, 1909, pp. 202 to 208).

Many histologists have laid great stress on the appearance of muscle examined in polarized light; but as the appearance of organic materials in polarized light depends on the direction and extent of strain to which they are subjected, it is doubtful just how these appearances ought to be interpreted. The writer has given elsewhere a more detailed discussion of the appearance of striated muscle in polarized light (Meigs, 1908*a*).

6. Portions of the structure of striated muscle which are not connected with contraction:

In addition to what might be called its contractile machinery, striated muscle contains such structures as are characteristic of all cells, as also structures which connect it with other parts of the body and make it a part of the organic community. It is outside the scope of this chapter to give any detailed account of these structures; they will be catalogued and described in the briefest way, but with references to the much fuller accounts which are to be found in the literature.

The nuclei of mammalian muscle are scattered along the fibers at irregular intervals immediately beneath the sarcolemma. They are elongated bodies with a length of about 10μ and a tranverse diameter of about 5μ. Each is usually surrounded with a small amount of undifferentiated cytoplasm. In many of the lower vertebrates, they are found in the interior of the fiber as well as along the surface.

The motor nerves of the muscle fibers pass through the sarcolemmas, and end in characteristic plates immediately beneath those membranes. The capillary blood vessels are very numerous, but run in the spaces between the fibers without entering them.

The connective tissue consists of a fairly thick sheath surrounding the whole muscle, from which small fibers and membranes pass into the interior, dividing it into bundles of fibers of various sizes, and finally forming delicate divisions between the individual fibers.

Descriptions of the structures mentioned above are to be found in Bailey (1925), Piersol (1920), and the other standard textbooks of histology.

Structures in striated muscle which may be homologous to the Golgi apparatus of nerve and gland cells have been described by several investigators whose work has been reviewed and cited by Pappenheimer (1916, pp. 129 and 130). The presence of mitochondria in developing muscle has been described by Gaudissart (1919 to 1920).

7. The pathology of striated muscle:

Striated muscle may undergo pathological changes as the result of various causes, such as mechanical and chemical injury, disturbances of the circulation, wasting diseases, abnormal growths, bacteria and animal parasites. As the result of such changes the fibers may be altered in size, may become necrotic, and may undergo various kinds of striking transformations in their appearance. It is outside the scope of this chapter to describe these changes or even to catalogue them in any detail. Descriptions of them with references to the original literature may be found in the standard textbooks of pathology (Delafield and Prudden, 1925; Stengel and Fox, 1921).

8. *The osmotic properties of striated muscle in relation to its function:*

When frog's striated muscle is immersed in distilled water, it swells, at first rather rapidly and then more slowly, and remains swollen. At the same time it shortens slowly, and remains shortened as long as it is left in the distilled water. If, however, it be transferred to 0.7 per cent sodium chloride solution, it slowly loses weight and, at the same time, slowly lengthens until it has returned to its original weight and length.

These facts certainly seem, at first sight, to confirm the view of the nature of contraction which was suggested in the preceding pages. For it is highly probable that the sarcostyles are involved in the swelling of the whole muscle which takes place in distilled water and in the subsequent loss of weight in the salt solution; and the hypothesis in question would be fully confirmed if it could be shown that the sarcostyles always shortened when their volume was artificially increased, and lengthened again when the artificial increase in volume was removed. But, unfortunately, this cannot be shown in such experiments as that which has just been described. For a whole muscle is composed, not only of sarcostyles, but also of sarcoplasmic spaces and of lymph spaces between the fibers. The whole muscle, therefore, may undergo changes in volume while the sarcostyles remain unchanged; or the sarcostyles may undergo changes in volume, while the whole muscle remains unchanged; and the significance of such experiments as that which has just been described depends on our knowledge of the osmotic properties of muscle and of its various parts. The present section will deal with this phase of the physiology of muscle.

The animal body is largely made up of complicated mixtures and combinations of proteins, water and salts. In some cases, as in that of the lymph, the water-protein-salt mixture is frankly a liquid; in other cases, as in that of tendon, the combination assumes the properties of a very tough and cohesive, though flexible and somewhat elastic solid. Striated muscle is a conglomeration of solid and liquid elements.

Practically all of the soft tissues of the body will increase in weight and volume if they are placed in distilled water, or will decrease in weight and volume if placed in certain kinds of salt solutions. The changes in weight and volume undergone by muscle in distilled water and in solutions of salts, sugars and other chemical compounds have been investigated in great detail; and there has been a disagreement among investigators as to the nature of these phenomena.

There are two possible reasons why a muscle immersed in distilled water might gain weight. It is conceivable that the muscle fibers might be surrounded by semi-permeable membranes, within which was a solution in which salts and other crystalloids were dissolved in such concentration as to give to this solution an osmotic pressure equal to that of the lymph. If, under these circumstances, the muscle were placed in distilled water,

salt would diffuse from the lymph spaces between the fibers out into the water, the osmotic pressure of the fluid surrounding the fibers would become less than that of the fluid within them, and water would pass from the surrounding spaces into the fibers. It is conceivable, on the other hand, that the muscle fibers are colloid threads of solid or semi-solid material, the surfaces of which are readily permeable to salts. If, on this hypothesis, the muscle were placed in distilled water, salts would diffuse out of the colloid material of the fibers into the surrounding liquid, and, as a consequence, the fibers would tend to take up water. It has been shown in a number of investigations that the tendency of such colloids as gelatin and fibrin, whether in solution or in the gel form, to take up water is inhibited by neutral salts (Moore and Parker, 1902; Lillie, 1907; Fischer, 1908).

It is worth while to consider these two hypotheses regarding the swelling of muscle in distilled water in connection with what has already been set forth regarding its structure. There is every reason to think that the muscle sarcostyles are threads of solid or semi-solid colloid material; they must have enough tensile strength to support and lift the weights which can be supported and lifted by contracting muscle. The histological evidence points further to the view that the sarcoplasm is a liquid. In the case of the insect's wing muscle, it does not prevent the fresh sarcostyles from being teased apart; in the case of the vertebrate muscle, it appears as clear in histological preparations as do the spaces between the fibers which are filled with lymph.

An additional interesting fact in this connection is that the fiber of vertebrate striated muscle is surrounded by a membrane, called the sarcolemma, which is easy to demonstrate histologically.

Either of the two hypotheses under consideration may therefore apply to the swelling of vertebrate striated muscle in distilled water. The sarcostyles are probably colloid threads which might behave like pieces of gelatin or fibrin in distilled water and various solutions. The sarcolemma, on the other hand, may be a semi-permeable membrane which divides the liquid sarcoplasm from the somewhat different liquid contained in the lymph spaces.

Work bearing on the points under consideration has been carried out by a long series of investigators. In a discussion like this, which is mainly morphological in character, it is not possible to give the results in any detail, but it is necessary to give some of the conclusions which may be drawn from them. These may be stated as follows:

The striated muscle fibers are made up of gelatinous sarcostyles and liquid sarcoplasm, and are surrounded by semi-permeable membranes. They are separated from each other by spaces filled with lymph. Both the sarcoplasm and the lymph contain salts in solution, and the osmotic pressure of the two solutions is equal. But the salts dissolved in the sarcoplasm

are quite different from those in the lymph. Potassium phosphate is the chief salt of the former, while sodium chloride is the chief salt of the latter. The semi-permeable membranes surrounding the fibers offer a high degree of resistance to the passage of these two salts and to that of many other soluble crystalloids.

Most of the protein of the muscle is contained in the sarcostyles, and the protein and water of the sarcostyles are combined in such a way that a large part of the latter cannot act as a solvent for salts.

The semi-permeable membranes surrounding the muscle fibers are easily destroyed by the action of distilled water as well as by that of other reagents. After they are destroyed, the muscle no longer responds to the osmotic pressure of solutions in which it is immersed, but acts a good deal as does a piece of gelatin or fibrin. It tends to swell in solutions of acids and alkalies and to lose fluid in solutions of neutral salts.

The evidence for the views outlined in the three preceding paragraphs has already been given by the writer (Meigs, 1912*b*).

The ability of striated muscle to contract in response to the electric current depends not only on the integrity of the semi-permeable membranes surrounding its fibers, but also on the presence of certain salts on the opposite sides of these membranes. If the membranes are destroyed by the action of distilled water, the muscle loses permanently its irritability to the electric current. If it be immersed in isotonic* sugar solution, it soon loses its power of responding to electrical stimulation, but this may be restored by transferring it to an isotonic sodium chloride solution (Overton, 1902, pp. 352 et seq.). But perhaps the most interesting point in this connection is that the irritability of the muscle may be temporarily abolished by immersing it in a potassium phosphate solution. Potassium phosphate is the chief salt contained in solution in the sarcoplasm, and it is a significant fact that the irritability of the muscle will not persist when the same salt is present on the two sides of the membranes which surround its fibers. Muscles which have been rendered temporarily unirritable by immersion in isotonic potassium phosphate solution are readily restored to irritability by transfer to isotonic sodium chloride solution (Overton, 1904, p. 199).

From the foregoing discussion, it will be seen that the swelling of vertebrate striated muscle in various reagents is a rather complicated process, and that swelling of the muscle as a whole need not necessarily mean any increase in the volume of the sarcostyles. It is quite conceivable that in the case of muscles immersed in moderately hypotonic solutions, fluid might enter the sarcoplasmic spaces and increase the volume of the fibers without

* By an *isotonic* solution in this discussion is meant a solution which has the same osmotic pressure as the lymph of the animal under consideration. A hypertonic solution means one with an osmotic pressure higher than that of the lymph; and a hypotonic solution, one with an osmotic pressure lower than that of the lymph.

affecting that of the sarcostyles. The supposition that shortening of the sarcostyles was the immediate mechanical result of their distention would not therefore necessarily be contradicted if it could be shown that the muscle as a whole might be caused to increase in volume without undergoing any shortening. The writer (1908*b*, p. 498) has shown that frog's striated muscle may be made to increase considerably in volume without undergoing any marked shortening by immersing it in half strength Ringer's solution.*

It has been possible, however, to bring forward a good deal of evidence for the view that the sarcostyles of vertebrate striated muscle have the property of shortening when their volume is artificially increased and lengthening when it is decreased. This evidence has consisted in a study of the changes of weight and length undergone by frog's striated muscle in various solutions after its semi-permeable membranes and irritability to the electric current have been destroyed by the action of distilled water. Under these circumstances, swelling of the tissue is accompanied by shortening, and decrease in volume by lengthening, provided that the reagents used are not of such a character as would be likely to destroy its structure altogether. For the results in detail, the reader is referred to the original article (Meigs, 1910).

Hürthle, (1909, pp. 122 to 130) reports an observation tending in the same direction, which was made on insect's leg muscle. He finds that the fibers of this form of muscle may be dried in a sulphuric acid desiccator at about $-15°$C., and that, if they are then immersed in glycerin, they assume an appearance closely resembling that of the uncontracted living fibers. If, however, such fibers are placed in water or Ringer's solution, they swell more or less, and at the same time shorten to about half their previous length and undergo all the miscroscopic changes which occur in the living contracting fibers. To the writer these observations seem to constitute very strong evidence for the view that the sarcostyles of striated muscle are so constructed that shortening is the immediate mechanical result of their distention.

9. *Relation between the structure of striated muscle and the biochemistry of contraction:*

The hypothesis that the contraction of striated muscle is the result of the swelling of its sarcostyles was proposed by McDougall, now nearly thirty years ago (McDougall, 1898). It is interesting that McDougall's histological studies combined with the very meager knowledge of the biochemistry of muscular contraction which existed at that time led him to

* Ringer's solution is a mixture of the chlorides of sodium, potassium and calcium with a small amount of sodium bicarbonate. It has approximately the same salt content as the lymph, and surviving frog's muscle immersed in it will retain its irritability for a long time.

suppose further that the swelling of the sarcostyles which resulted in their shortening was brought about by the production of lactic acid within the muscle (McDougall, 1898). Since the publication of McDougall's articles a great mass of evidence has accumulated, which makes it possible to connect our knowledge of the structure of muscle with our knowledge of the biochemistry of contraction in a good deal of detail. The connection between these two fields of knowledge may be sketched as follows:

As has just been pointed out, striated muscle is made up of colloid sarcostyles surrounded by fluid sarcoplasm, in which the chief salt dissolved is potassium phosphate. The sarcoplasmic fluid with its dissolved potassium phosphate is separated from the lymph contained in the spaces between the muscle fibers by the semi-permeable sarcolemmas surrounding them. The lymph contains considerable quantities of sodium bicarbonate in solution.

As the reaction of muscle is nearly neutral, the potassium phosphate contained within the fibers must be a mixture of mono-and di-potassium phosphate. Such phosphate mixtures are typical buffer solutions; they have the property of remaining nearly neutral even when considerable quantities of weak acid are added to them. Sodium bicarbonate solutions are also typical buffers; when acid is added to them, it combines with the sodium, displacing carbonic acid, which tends to escape as carbon dioxide. The important part played by buffers in various physiological activities has been studied by Henderson (1909).

Striated muscle, then, may be regarded as a system of fine colloid threads, which have the property of shortening when distended, and which are surrounded by a double defense of buffer solutions. Many colloids have the property of taking up fluid from surrounding solutions when their reaction is made more acid (Pauli, 1912; Fürth, 1919), and there is reason to believe that the sarcostyles of striated muscle also have this property (McDougall, 1897, 1898; Fischer, 1908). We have, therefore, in striated muscle a system in which the production of a small amount of acid would be followed by swelling and shortening of the sarcostyles. There would be, however, an almost immediate tendency for the acid to be neutralized by the buffer salts of the muscle and for the consequent reversal of the swelling and shortening of the sarcostyles.

What is known of the biochemistry of contraction fits in very well with the above account of the structure of muscle and of the physical chemistry of its various parts. It has been shown that vertebrate striated muscle will contract and relax again repeatedly, even when entirely deprived of oxygen. Under these circumstances, the chief chemical change that goes on within it is the production of lactic acid from carbohydrate and its neutralization by the buffers of the muscle fluids. When muscle deprived of oxygen is repeatedly stimulated, lactic acid in the form of lactate accumulates

within it up to the quantity of about 0.2 per cent of its weight, and the muscle then becomes entirely unirritable. It still remains relaxed, however, and its reaction is very nearly neutral.

Two different courses may now be taken with the muscle. It may be heated to 45°C., in which case much more lactic acid is formed within it, its reaction becomes distinctly acid, and it goes into the permanent contraction of rigor and never recovers its irritability. Or it may be supplied at room temperature with plenty of oxygen, in which case, the lactate contained within it tends to disappear, much carbon dioxide is evolved, and irritability is recovered.

What is known of the biochemistry of contraction, therefore, fits in perfectly with the view suggested by a study of the structure of muscle, the view, namely, that the only chemical changes immediately necessary to bring about contraction and relaxation are the production of lactic acid within the muscle and its neutralization by the buffer salts. By some biochemical arrangement not yet understood the muscle loses its irritability to stimulation by the electrical current before enough lactic acid has been formed to overcome the neutralizing action of the buffers and cause permanent swelling and shortening of the sarcostyles. Further lactic acid formation and permanent swelling and shortening of the sarcostyles may, however, be brought about at this point by heating the muscle; or the lactate already present may be removed and irritability may be restored by supplying oxygen and giving an opportunity for the oxidative changes which are a normal part of the chemical activity of the muscle, but not of those changes which are directly connected with contraction.

Detailed accounts of the chemistry of contraction in vertebrate striated muscle with references to the literature have been given by Hill and Meyerhoff (Hill and Meyerhoff, 1923; Meyerhoff, 1924). Accounts connecting lactic acid formation with the imbibition theory of contraction have been given by Pauli (1912), and Fürth (1919).

II. SMOOTH MUSCLE

1. *The structure of vertebrate smooth muscle:*

Vertebrate smooth muscle is made up of fiber cells which differ very markedly from the fiber cells of striated muscle. The former cannot be teased apart in fluids such as Ringer's solution, in which the tissue remains alive; and the descriptions which have been given of the fresh tissue are, therefore, rather vague. Engelmann (1881, p. 546), however, has stated that the fresh fibers of vertebrate smooth muscle appear homogeneous, showing no sign of either longitudinal or cross striation, and the same statement is made by Lewis ("General Cytology" p. 396) regarding living smooth muscle cells in tissue cultures.

In histological preparations, the outlines of the smooth muscle fibers vary greatly in distinctness. It is often nearly impossible to make out the boundary line between a cell and the surrounding interstitial substance. In certain preparations, however, this can be done more easily, and it can then be made out that the smooth muscle fibers are very much smaller than those of striated muscle. The former seldom reach a diameter of more than 10μ in vertebrates, while the fibers of vertebrate striated muscle are from 50 to 100μ in diameter on the average. The nuclei of the smooth muscle fibers are elongated bodies. Each fiber cell has only one nucleus situated at its middle, and occupying, at that level, a large proportion of the diameter of the cell.

Heidenhain (1900, pp. 213 and 214) and a number of other histologists have endeavored to find in smooth muscle fibrillae or sarcostyles corresponding to those of striated muscle, but the appearance of the two tissues in similar histological preparations gives little basis for such a supposition. Figure 348 is the reproduction of a photograph by ultraviolet light of a longitudinal section of the uncontracted smooth muscle of the frog, and is to be compared with Figure 340, which shows a similar preparation of striated muscle. Figure 349 shows a transverse section of frog's smooth muscle to be compared with Figure 342, showing the striated muscle of the same animal.

The smooth muscle shown in Figures 348 and 349 was fixed in Zenker's fluid, while the striated muscle shown in Figures 340 and 342 was fixed in 70 per cent alcohol. The writer has not been able to compare uncontracted preparations of smooth and striated muscle fixed with the same reagent, because all of the ordinary fixatives cause a marked contraction in one or the other or both of the two tissues. The results have been controlled as far as possible by comparing preparations of contracted smooth muscle fixed, on the one hand, in 70 per cent alcohol, and on the other hand, in Zenker's fluid, and determining that the appearance of the contracted tissue is about the same, whichever of the two fixatives is used (Meigs, 1908*b*). The formula for the Zenker's fluid used is to be found in Lee's textbook (1913, p. 50).

The wide difference in the character of the two tissues appears very clearly from a study of Figures 340, 342, 348 and 349. While the fibers of the striated muscle are evidently made up of definite elements which have a definite structure and arrangement, those of the smooth muscle show nothing but an irregular appearance of vague lines and dots. The lines have, perhaps, a generally longitudinal direction in the longitudinal section, but the appearance of Figures 348 and 349 is, on the whole, suggestive of the appearances which Fischer (1899) has described in precipitated hemoglobin and coagulated blood serum.

The chief indications which show that the sarcostyles "pre-exist" in striated muscle fail when it comes to a question of the "fibrillae" in smooth

muscle. One form of striated muscle may be teased, while still living, into its constituent sarcostyles; no such form of smooth muscle is known. In all forms of striated muscle, the living fibers appear plainly longitudinally striated; the fresh smooth muscle fibers show no longitudinal striation. A very small amount of histological treatment makes possible the teasing of

348

349

FIG. 348.—Longitudinal 1μ thick of the uncontracted smooth muscle of the frog. Zenker fixation; paraffin, glycerin. a, a, nuclei. × 1800.

FIG. 349.—Transverse section 1μ thick of the uncontracted smooth muscle of the frog. Zenker fixation; paraffin; glycerin. a, a, nuclei. × 1800.

the striated muscle fibers into their sarcostyles; no one has by any method succeeded in teasing the smooth muscle fibers into similar elements. The histological evidence, therefore, indicates that smooth muscle has a much

simpler structure than striated muscle, being made up of homogeneous colloid threads which have a certain amount of interstitial substance between them.

2. *Changes undergone by the smooth muscle fibers during contraction:* The question of the different forms assumed by smooth muscle fibers in relaxation and in contraction has been touched on by Henneberg and by Heiderich. Henneberg (1901) asserts that the contracted fibers stain less deeply and show more signs of fibrillation. The argument by which Henneberg tries to show that one type of fiber is contracted and the other relaxed is not very convincing. He gives an extended description of his methods for obtaining muscle in contraction and relaxation, but ends by saying that both types of fibers were observed in all the specimens. In various other ways the article is somewhat unsatisfactory. The author states that he made use of a number of methods in fixing the tissue, and finally found heat to be the best; he used this method to fix his tissue in relaxation. He does not say however, whether he was careful, in making his comparisons, to use relaxed and contracted tissue fixed by the same method. There is reason to believe that heat may sometimes produce a marked shortening in smooth muscle (Vernon, 1899).

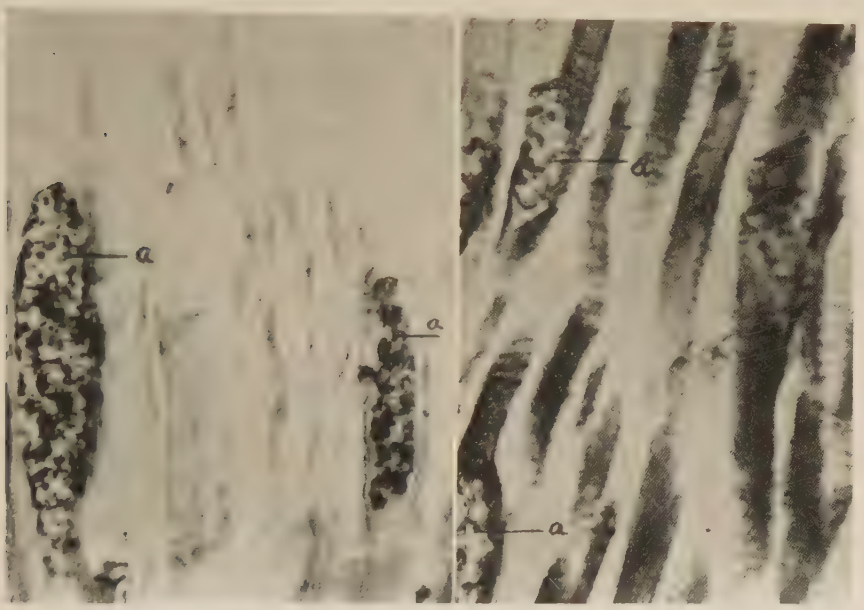
Heiderich (1902) comes to just the opposite conclusion from Henneberg in regard to the appearance of preparations of uncontracted and contracted smooth muscle. He finds that the uncontracted fibers stain less deeply and show more signs of fibrillation than do the contracted ones.

The writer (1908*b*) has made a study of the effects of fixing reagents on smooth muscle, and of the appearances presented by the tissue when fixed, on the one hand, in relaxation, and, on the other, in contraction. Pieces of smooth muscle immersed in 70 per cent alcohol contract markedly and are fixed in contraction; while Zenker's fluid (Lee, 1913, p. 50) fixes the tissue at the length which it happens to have when immersed.

Smooth muscle may be obtained in both the relaxed and contracted conditions by cutting off short sections from the small intestine of freshly killed amphibians. The circular muscle of the intestine contracts near the cut ends of such sections, while that in the middle remains relaxed. If such pieces of intestine are thrown immediately into Zenker's fluid, the circular muscle near the ends is fixed in contraction; and that at the middle, in relaxation. Figures 350 and 351 are longitudinal sections of muscle in relaxation and contraction respectively, obtained from such preparations of the intestine of *Necturus;* and Figures 352 and 353 are corresponding cross sections.

Study of the figures shows that in the contracted muscle the fibers stain more deeply, and lie farther apart than in the uncontracted muscle. They have about the same diameter whether contracted or uncontracted. The

nuclei in the contracted muscle are shorter and rounder, and the fibers are more bulged out at their levels. The differences between the uncontracted and contracted muscle could readily be explained by supposing that, during contraction, the fibers give up fluid to the interstitial spaces. Such a change would result in greater concentration of the protein material contained in the contracted fibers, and thus account for their deeper staining. It would also account for the large interstitial spaces in the contracted muscle and the failure of the fibers to increase noticeably in diameter during contraction.



350 351

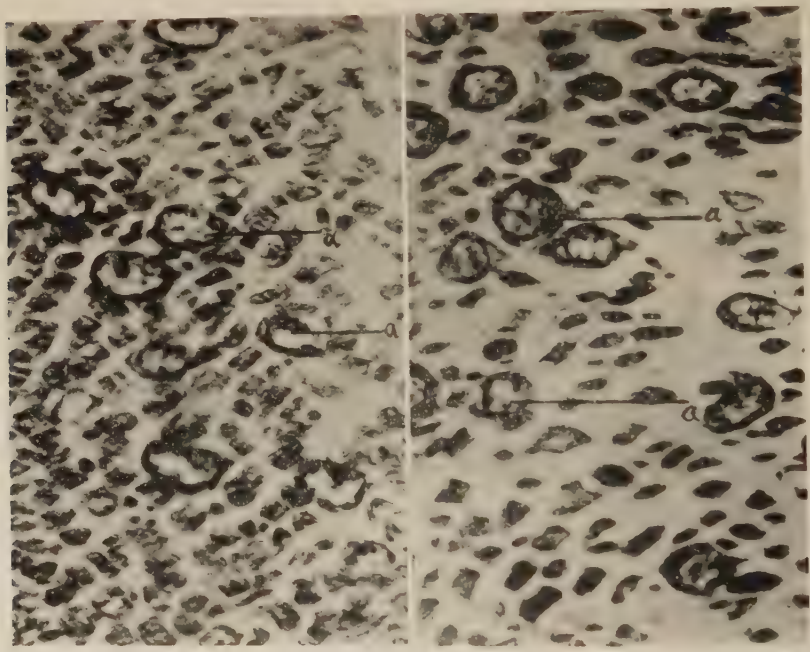
FIG. 350.—Longitudinal section 3μ thick of the uncontracted smooth muscle of necturus. Zenker fixation; paraffin; Canada balsam; Mallory's stain. a, a, nuclei. × 1000.

FIG. 351.—Longitudinal section 3μ thick of the contracted smooth muscle of necturus. Zenker fixation; paraffin; Canada balsam; Mallory's stain. a, a, nuclei. × 1000.

Heiderich (1902), has given a description similar to the above of the differences between histological preparations of relaxed and contracted smooth muscle. He supposes, however (1902, p. 475), that the different appearances are to be accounted for by assuming that the contracted smooth muscle fibers shrink more under the influence of histological treatment than do the uncontracted fibers. It is interesting to compare this assumption of Heiderich's with a somewhat similar assumption made by Gutherz (1910) in regard to the appearance of cross sections of relaxed and contracted striated muscle. Gutherz found, as has the writer, that in the

case of cross sections of striated muscle the area occupied by the sarcostyles is larger in proportion to the sarcoplasmic spaces when the tissue is contracted. He assumes, therefore, that the contracted sarcostyles shrink less under the influence of histological treatment than do the uncontracted ones.

It seems rather gratuitous to suppose that histological treatment causes more shrinkage in the contracted than in the uncontracted elements of smooth muscle, and less, in the contracted than in the uncontracted ele-



352 353

FIG. 352.—Transverse section 3μ thick of the uncontracted smooth muscle of necturus. Zenker fixation; paraffin; Canada balsam. Mallory's stain. a, a, nuclei. × 1000.

FIG. 353.—Transverse section 3μ thick of the contracted smooth muscle of necturus. Zenker fixation; paraffin; Canada balsam; Mallory's stain. a, a, nuclei. × 1000.

ments of striated muscle. In the case of smooth muscle, it is possible, fortunately, to show that the contracted fibers occupy a smaller volume in relation to the interstitial spaces than the uncontracted ones in preparations of fresh living tissue. Smooth muscle can be cut into fairly thin cross sections without destroying its irritability, and the study of such sections of relaxed and contracted muscle indicates that, during contraction, fluid passes from the fibers to the interstitial spaces (Meigs, 1912a).

As far as the writer knows, there has been no other serious attempt to determine from the study of surviving smooth muscle whether or not the fibers change in volume during contraction. Schultz has studied the surviving stomach muscle of *Salamandra maculosa*, and asserts that the fibers become thicker as they contract (1895, pp. 531 and 532). It would be extremely difficult, however, to determine from the study of such preparations as Schultz used whether or not the fibers became thicker during contraction, and quite impossible to determine whether they changed in volume during contraction.

3. *Portions of the structure of smooth muscle which are not directly connected with contraction:*

The nuclei, nerve endings and other structures of smooth muscle, which are not directly connected with contraction, will be only very briefly mentioned as in the case of striated muscle.

Each smooth muscle cell contains only one nucleus, which is an elongated body with a long diameter of about 20μ and a short diameter of about 5μ, situated near the middle of the cell. The appearance of the smooth muscle nuclei is fairly well shown in Figures 348 to 353.

The motor nerves end on the surfaces of the fibers without forming any such plates as are characteristic of striated muscle (Piersol, 1920, p. 86, Fig. 122). For further discussion of innervation see Hines, 1927.

The connective tissue consists of a system of membranes (Heidenhain, 1900, p. 157) or fibers (Bailey, 1925, p. 106) which not only divide the muscle fibers up into bundles, but also separate the individual fibers.

The capillary blood vessels of smooth muscle, like those of striated muscle, run chiefly in the same direction as the muscle fibers through the interstitial spaces without entering the fibers. The supply is much more meager than that of striated muscle (Piersol, 1920, p. 55).

4. *The osmotic properties of smooth muscle in relation to its function:*

The smooth muscle of the frog very generally lengthens in solutions which cause it to increase in weight and shortens in solutions which cause it to decrease in weight, and these facts are in harmony with the view that contraction in this tissue is the result of the loss of fluid by its fibers. As in the case of striated muscle, however, there is no necessary connection between changes in volume of the muscle fibers and changes in volume of the muscle as a whole; and the significance of the connection between changes in length and changes in volume will depend upon our knowledge of the osmotic properties of the tissue and of its various parts.

The histological evidence indicates that the structure of smooth muscle is simpler than that of striated muscle. In the case of the latter, we have the gelatinous and cohesive sarcostyles surrounded by the fluid sarcoplasm,

sarcostyles and sarcoplasm together being enclosed by the sarcolemma, and separated by it from the lymph contained in the spaces between the fibers. In the case of smooth muscle, on the other hand, we have only the fibers and the spaces between them. There is no satisfactory evidence for the existence of differentiated membranes surrounding the fibers.

A study of the changes of weight undergone by striated muscle in various solutions indicates that the sarcolemmas are semi-permeable membranes which are easily destroyed. Study of the changes of weight undergone by smooth muscle in similar solutions indicates that its osmotic properties are very different from those of striated muscle, and that it behaves, in many respects, like such colloids as gelatin and fibrin.

The writer has made a somewhat detailed study of the osmotic properties of the striated and smooth muscle of the frog (Meigs, 1921*b*), which may be summarized at this point.

Striated muscle maintains a nearly constant weight when immersed in a number of different solutions of both electrolytes and non-electrolytes, provided that these are neutral in reaction and have the same osmotic pressure as frog's blood. The behavior of smooth muscle immersed in such solutions, on the other hand, depends on the chemical nature of the solution rather than on its osmotic pressure. Smooth muscle, for instance, swells markedly in isotonic solutions of non-electrolytes. In Ringer's solution it swells slowly and remains swollen; in isotonic sodium chloride solution, it first gains weight and then loses; in isotonic potassium chloride solution, it first loses weight and then gains. Experiments with solutions which are not isotonic with frog's blood give further reason to believe that the surfaces of the smooth muscle fibers are not semi-permeable in the same sense as those of the striated muscle fibers. In double-strength Ringer's solution, striated muscle loses weight at first rapidly and then more slowly, and in half-strength Ringer's solution, it gains weight at first rapidly and then more slowly, the curves of gain and loss being similar in the two cases, except for the reversal of the direction. Smooth muscle, on the other hand, gains weight only very slowly in half-strength Ringer's solution hardly any faster, if at all, than in isotonic Ringer's solution. In double-strength Ringer's solution it loses weight quite rapidly, but the rate of loss increases in the early stages instead of diminishing from the beginning, as is the case with striated muscle.

There are further interesting differences in the way in which the two tissues react to various influences, which point in the same direction. Overton has brought out the important point that striated muscle will not remain irritable to the electrical current unless its fibers are surrounded with a solution which contains either sodium or lithium salts (Overton, 1902, 1904). The facts are such as to indicate that the irritability of the tissue depends on the presence of potassium salts within the semi-permeable membranes

surrounding the fibers and sodium salts outside of them. Smooth muscle, on the other hand, remains irritable for a long time in solutions of potassium salts and in sugar solutions, even though it may take up large quantities of fluid from such solutions.

But perhaps the most definite indication for the presence of semi-permeable membranes surrounding the fibers of striated muscle and for the absence of such membranes in the case of smooth muscle is the manner in which the two tissues respond to cuts across their fibers. Striated muscle goes almost immediately into rigor in the neighborhood of such a cut, and the shortening and opacity characteristic of rigor spread gradually from the neighborhood of the cut to other parts of the tissue. Smooth muscle, on the other hand, is little affected by cuts across its fibers; even quite thin cross sections of it will remain irritable to the electrical current for a long time in Ringer's solution.

In the preceding section, evidence was given for the view that increase in the volume of the sarcostyles of striated muscle caused them to shorten, and that the shortening which takes place in normal contraction was the result of an increase in the volume of the sarcostyles. In the present section, evidence has been given to show that the normal contraction of smooth muscle is accompanied by a decrease in the volume of its fibers, and it is an interesting question whether the decrease in the volume of the tissue as a whole, which may be brought about by immersing it in various solutions, and which may presumably be attributed chiefly to a decrease in the volume of its fibers, is accompanied by shortening.

The writer (Meigs, 1912*b*) has made a study of the changes of weight and length undergone by smooth muscle in Ringer's solution under various conditions, in distilled water and in solutions of various salts, sugars and other crystalloids. It was found that although the changes in weight of the muscle bore only a rough relation to the osmotic pressure of the solution in which it was immersed, nevertheless, it was a very general rule that increase in weight was accompanied by increase in length of the fibers, and decrease in weight, by decrease in length. The changes in weight and length were often roughly proportional to one another. In all the experiments, the changes in irritability undergone by the muscle were followed. It was found that this was sometimes maintained for a long time and sometimes rapidly lost, but whichever of these two alternatives was the case, the weight changes and the changes in length of the fibers went hand in hand. In other experiments it was shown that pieces of living smooth muscle tended to lose weight when they were made to contract by electrical, chemical or mechanical stimulation.

In a recent review of the subject of smooth muscle, Evans (1926, p. 386) states that a good deal of this work has been repeated in his laboratory and that, in general, the same results have been obtained. He doubts, however, whether there is any causal connection between change in volume

and change in length of the smooth muscle fibers, because the changes in length and weight of the muscle are not always proportional.

Such experiments, unfortunately, cannot go beyond determining the changes in weight which take place in the muscle as a whole, and these may not be exactly proportional to the changes in volume of the fibers, on account of the fact that it is always possible for the interstitial spaces also to change in volume. Failure of the changes in weight and length of the muscle to be exactly proportional under all conditions does not, therefore, necessarily show that there is no causal connection between changes in volume and length of the fibers. It is true, of course, that the general rule that increase in weight of the muscle is accompanied by lengthening is less strict proof of the causal connection between lengthening and increase of volume of the fibers than would be an invariable proportional relation; but the facts as they stand seem to the writer to constitute strong evidence for the view that such a causal relation exists.

On the whole, then, there is the same kind of general agreement between the histological appearance of smooth muscle in relaxation and contraction and the changes of weight and length which it undergoes in various solutions, as is the case with striated muscle. In the case of smooth muscle, the histological evidence indicates that the fibers lose fluid when they contract, and solutions which cause the muscle to decrease in volume generally also cause it to shorten. In the case of striated muscle, on the other hand, the histological evidence indicates that the sarcostyles take up fluid when they contract, and solutions which cause the muscle to increase in volume commonly also cause shortening. The exceptions to this rule receive a reasonable explanation from the complicated structure of the tissue.

5. The biochemistry of contraction in smooth muscle:

The biochemistry of contraction in smooth muscle has been very little studied, but there is some reason to believe that lactic acid formation plays an important part in this process. The writer (Meigs, 1909) has shown that small amounts of lactic acid are produced in smooth muscle when it is heated to between 40° and 50°C. amounts much smaller than those produced in striated muscle under similar circumstances. Evans (1926, p. 388) has shown that lactic acid is formed in smooth muscle when it is subjected to repeated stimuli.

It is a common rule that colloids swell more in acid than in neutral solutions; and, if the contraction of smooth muscle is caused by the loss of fluid from its fibers, as the results given up to this point seem to indicate, it is a little difficult, at first sight, to see how lactic acid formation could bring about this process.

It is not worth while to discuss at any length a question which has been so little investigated, but it may be pointed out that it is not impossible to

suppose that the uncontracted smooth muscle fibers are slightly alkaline. Under these circumstances, the formation of a small amount of acid would cause them to become more nearly neutral and consequently to give up fluid to their surroundings. Colloids have a tendency to swell in alkaline as well as in acid solutions.

The above is put forward only as a suggestion, but it is interesting in connection with it, that smooth muscle may be thrown into a state in which it lengthens in response to electrical stimulation, by keeping it for some time either in double strength Ringer's solution or in slightly acid Ringer's solution (Meigs, 1912*b*, pp. 509 and 546). It seems likely that stimulation would cause lactic acid production under these circumstances as well as in perfectly normal muscle; and, if this is the case, it would appear that the colloids of the smooth muscle fibers may easily be shifted from a condition in which they give one response to lactic acid formation to a condition in which they give the opposite response.

Similarities and differences in the biochemistry of smooth and striated muscle are discussed in some detail by Evans (1926), and references are given to the literature. Like striated muscle, smooth muscle produces lactic acid more readily in the presence of alkali and in the absence of oxygen. In general, however, its chemical activity is much less than that of striated muscle; not only is the lactic acid production smaller, but also the oxygen consumption and the heat output. A further very interesting difference between the two tissues lies in the power of smooth muscle to remain for indefinitely long periods in a state of marked contraction or tone without any increase in either lactic acid production, oxygen consumption or carbon dioxide output. In the case of striated muscle, the maintenance of a state of marked contraction under normal conditions is always accompanied by a maximal chemical activity.

III. CONCLUDING REMARKS

It seems to the writer by far the most probable hypothesis of muscular contraction that the changes in length of the muscular elements are the mechanical result of changes in volume, and that these changes in volume are brought about by changes in the hydration of the muscle colloids, which again are caused by the production and neutralization of lactic acid. It seems worth while in closing to summarize the connection between this hypothesis and the observations on which it is based, and to point out that the whole group of observations will have a certain independent importance for histologists, even if it should turn out that the hypothesis toward which they seem to point is illusory.

There is very good general agreement at present that the production and neutralization or disappearance of lactic acid are the chief chemical

events which bring about the contraction and relaxation of striated muscle. What is known of the physiology and biochemistry of this tissue fits in well with the view that contraction is the result of an increase in hydrogen-ion concentration within the muscle; and relaxation, the result of a decrease. There is no doubt, further, that the hydration of many colloids, and of the muscle colloids among the others, is greatly increased, under certain circumstances, by small increases in their hydrogen-ion concentration.

Further evidence on the subject is to be obtained along two lines. There is first the question whether the muscle fibrils or sarcostyles can be shown by histological means to increase in volume when they contract, and second the question whether there is any significant relation between changes in weight and length of the muscle as a whole, when it is subjected to the action of swelling reagents and their opposites.

The evidence on the first question is somewhat conflicting. Ecyleshymer, Hürthle, Gutherz and the writer have made observations on the cross sections of muscle fibers fixed in relaxation and contraction which would indicate that during contraction fluid passes from the sarcoplasmic spaces to the sarcostyles; Marcus, from measurements of fixed muscle, comes to the opposite conclusion. The writer's measurements of the uncontracted and contracted sarcostyles of the surviving wing muscle of the fly would indicate that these structures increase in volume during contraction, while Hürthle's conclusions from measurements of his cinematographic photographs are, to some extent, opposed to this view. To the writer, the weight of evidence seems definitely in favor of the view that the sarcostyles increase in volume during contraction, but the subject is a very difficult one, and it will perhaps be some years yet before it is satisfactorily decided.

It may be definitely stated that striated muscle may be caused to shorten markedly by immersing it in certain swelling reagents, and that the shortening so caused may be removed by transferring it to certain reagents which cause it to lose fluid. It is not, however, an invariable rule that shortening goes hand in hand with increase in volume of the muscle as a whole. Many reagents cause such increases in volume without any accompanying shortening.

The structure of the muscle, however, is so complicated that it is not to be expected that an increase in the volume of the whole muscle would always mean an increase in the volume of the sarcostyles, and the hypothesis outlined at the beginning of this discussion does not, therefore, demand any strict parallelism between changes in length and changes in volume of the muscle as a whole.

The complicated structure of the muscle makes it impossible finally to prove or disprove the hypothesis in question by studying the relation between changes in length and in volume of the whole muscle. But such

observations as those of Hürthle (1909, pp. 122 to 130) and the writer (1910), which show that dead muscle may often be made to shorten markedly by increasing its volume, constitute strong evidence in favor of the hypothesis. For a structure which shortens markedly when its volume is increased is complicated and unusual, and it is unlikely that it would be present in muscle unless it had something to do with its function.

In the case of smooth muscle, the chemical changes which accompany contraction have been much less studied than in the case of striated muscle, but here also there is some evidence that the production of lactic acid from carbohydrate plays a leading part. In smooth muscle, therefore, as well as in striated muscle, the chief chemical change accompanying contraction is apparently of such a nature as to change the hydrogen-ion concentration and with it the hydration of the tissue colloids.

In the case of smooth muscle, the evidence that the tissue elements change in volume during contraction and that the tissue may be artificially made to lengthen or shorten by immersing it in swelling or in dehydrating solutions is more complete and satisfactory than in the case of striated muscle. A decrease in volume of the smooth muscle cells during contraction can be demonstrated not only in fixed preparations, but also in the surviving tissue; and there is almost no contradictory evidence. Further, the relation between lengthening and increase in volume of the tissue as a whole is very striking, and the exceptions to this rule, which have so far been observed, are not of such a nature as to disprove the view that changes in volume are the cause of changes in length of the individual cells.

Even if it should turn out finally that changes in volume of the muscle elements did not always accompany contraction and were not really the cause of it, nevertheless, the observations on which the above-outlined hypothesis rests would have to be reckoned with by histologists as well as by physiologists. For they show without any question that the muscle elements are made up of colloids which readily exchange fluid with their surroundings, that acid is likely to be produced in muscle under both normal and abnormal conditions, and that a change in hydrogen-ion concentration has a powerful effect in bringing about a change in the tendency of the muscle colloids to take up and hold water. Whether changes in the hydration of the muscle colloids are the cause of contraction or not, therefore, there can be no doubt that they are likely to take place in surviving muscle both before and during its subjection to histological reagents, and to influence the microscopic appearance of the tissue.

DETAILS REGARDING THE ILLUSTRATIONS

All of the illustrations except Figure 335 are reproduced from those given in two articles by the writer (Meigs, 1908*a*, 1908*b*). Figures 336 to 347 inclusive are from the first named of these articles. They are all reproductions of photographs taken by ultra-

violet light at the Zeiss works at Jena, and in all cases the tissue used was unstained. Figure 336 shows fresh tissue teased out in 0.7 per cent sodium chloride solution; Figures 343 to 345, fresh tissue teased out in a mixture of equal parts of white of egg and 2 per cent sodium chloride solution; Figure 339, tissue fixed in 70 per cent alcohol and teased out in glycerin. All of the other figures in this group, namely 337, 338, 340, 341, 342, 346 and 347 show tissue which was fixed in 70 per cent alcohol, embedded in the usual way in paraffin, cut into sections, and finally preserved in glycerin.

Figures 348 to 353 inclusive are from the second of the two above-mentioned articles. The tissue shown in Figs. 348 and 349 was prepared and photographed in every way like that shown in Figures 340 and 342, except that it was fixed in Zenker's fluid (Lee, 1913, p. 50) instead of in 70 per cent alcohol. The tissue shown in Figs. 350 to 353 inclusive was fixed in Zenker's fluid, embedded in the usual way in paraffin, cut into sections, stained with Mallory's phosphotungstic acid hematoxylin (Mallory, 1905, p. 116) and preserved in Canada balsam. Figures 348 and 349 show the intestinal muscle of the frog; Figures 350 to 353 inclusive, that of necturns.

The writer wishes to express his thanks to the publishers of the *Zeitschrift für allgemeine Physiologie* and of the *American Journal of Physiology* for permission to reproduce the illustrations.

IV. BIBLIOGRAPHY

Bailey, F. R. 1925. *Text-book of histology*, revised and rewritten by O. S. Strong and A. Elwyn. Ed. 7, New York: W. Wood & Co.

Biedermann, W. 1909. Vergleichende Physiologie der irritablen Substanzen. *Ergeb. d. Physiol.*, **8**, 26.

Delafield, F., and Prudden, T. M. 1925. *Text-book of pathology*. Ed. 13, New York: W. Wood & Co.

Engelmann, T. W. 1881. Ueber den faserigen Bau der contractilen Substanzen, mit besonderer Berücksichtigung der glatten und doppelt schräggestreiften Muskelfasern. *Pflüger's Arch. ges. Physiol.*, **25**, 538.

Evans, C. L. 1926. The physiology of plain muscle. *Physiol. Reviews*, **6**, 358.

Eycleshymer, A. C. 1904. The cytoplasmic and nuclear changes in the striated muscle cell of Necturus. *Am. J. Anat.*, **3**, 285.

Fischer, A. 1899. *Fixierung, Färbung, und Bau des Protoplasmas*. Jena: Gustav Fischer.

Fischer, M. H. 1908. Ueber die Analogie zwischen der Wasserabsorption durch Fibrin und durch Muskel. *Pflüger's Arch. ges. Physiol.*, **124**, 69.

Fürth, O. 1910. Die Kolloidchemie des Muskels und ihre Beziehungen zu den Problemen der Kontraktion und der Starre. *Ergeb. d. Physiol.*, **17**, 363.

Gaudissart, P. 1910 20. Reseau protoplasmique et chondriosomes daus la genèse des myofibrilles. *La Cellule*, **30**, 29.

Gutherz, S. 1910. Zur Histologie der quergestreiften Muskelfaser insbesondere über deren Querschnittsbild bei der Kontraktion. *Arch. f. mikr. Anat. u. Entw.*, **75**, 209.

Haycraft, J. B. 1891. On the minute structure of striped muscle with special reference to a new method of investigation by means of impressions stamped in collodion. *Pro. Royal Soc.*, London, **49**, 287.

Heidenhain, M. 1898. Struktur der kontraktilen Materie. 1. Struktur der quergestreiften Muskelsubstanz. *Anat. Hefte* (Ergeb. Anat., etc.) **8**, 3.

——— 1900. Struktur der kontraktilen Materie. *Ibid.*, **10**, 115.

Heiderich, F. 1902. Glatte Muskelfasern im ruhenden und thätigen Zustande. *Anat. Hefte*, **19**, 449.

Henderson, L. J. 1909. Das Gleichgewicht zwischen Basen und Säuren im tierischen Organismus. *Ergeb. d. Physiol.*, **8**, 254.

Henneberg, B. 1901. Ruhende und thätige Muskelzellen in der Arterienwand. *Anat. Hefte*, **17**, 425.

Hill, A. V., and Meyerhoff, O. 1923. Ueber die Vorgänge bei der Muskelkontraktion. *Ergeb. d. Physiol.*, **22**, 299.

Hines, M. 1927. *Quart. Rev. Biol.*, **2**, 149.

Hürthle, K. 1907. Ueber die Struktur des quergestreiften Muskels in ruhenden und tätigen Zustande, und über seinen Aggregatzustand. *Biol. Centralbl.*, **27**, 112.

——— 1909. Ueber die Struktur der quergestreiften Muskelfasern von Hydrophilus im ruhenden und tätigen Zustand. *Pflüger's Arch. ges. Physiol.*, **126**, 1.

——— 1914. Erwiderung auf die vorliegende Ansicht von Meigs. *Ibid.*, **158**, 100.

Janet, C. 1907. *Anatomie du corselet et histolyse des muscles vibrateurs, après le vol nuptial, chez la reine de la fourmi (Lasius niger)*. Limoges, France: Ducourtieux et Gont.

Janisch, E. 1923–24. Zum Bau der quergestreiften und glatten Muskelsäulchen, nach Untersuchungen an Bombus terrestris und Helix pomatia. *Anat. Anz.*, **57**, 246.

Lee, A. B. 1913. *The microtomist's vade-mecum*. Phila.: P. Blakiston's Son & Co.

Lillie, R. S. 1907. The influence of electrolytes and of certain other conditions on the osmotic pressure of colloidal solutions. *Am. J. Physiol.*, **20**, 127.

McDougall, W. 1897. On the structure of cross-striated muscle and a suggestion as to the nature of its contraction. *J. Anat. and Physiol.*, **31**, 410, 539.

——— 1898. A theory of muscular contraction. *Ibid.*, **32**, 187.

Mallory, F. B. 1905. A contribution to the classification of tumors. *J. Med. Res.*, **13**, 113.

Marcus, H. 1919–21. Ueber den feineren Bau quergestreifter Muskeln. *Arch. Zellf.*, **15**, 393.

Meigs, E. B. 1908*a*. The structure of the element of cross-striated muscle, and the changes of form which it undergoes during contraction. *Ztschr. f. allg. Physiol.*, **8**, 81.

——— 1908*b*. The application of McDougall's theory of contraction to smooth muscle. *Am. J. Physiol.*, **22**, 477.

——— 1909. Heat coagulation in smooth muscle; a comparison of the effects of heat on smooth and striated muscle. *Ibid.*, **24**, 1.

——— 1910. The effects of distilled water and of various solutions on the weight and length of striated muscle. *Ibid.*, **26**, 191.

——— 1912*a*. Microscopic studies of living smooth muscle. *Ibid.*, **29**, 317.

——— 1912*b*. Contributions to the general physiology of smooth and striated muscle. *J. Exper. Zool.*, **13**, 497.

——— 1914. Ob die Fibrillen der quergestreiften Muskeln ihr Volum während der Kontraktion verändern? *Pflüger's Arch. ges. Physiol.*, **158**, 92.

Merkel, F. 1872. Der quergestreifte Muskel. 1. Das primitive Muskelelement der Arthropoden. *Arch. mikr. Anat.*, **8**, 244.

Meyer, A. 1921. *Analyse der Zelle*. Jena: Gustav Fischer.

Meyerhoff, O. 1924. *Chemical dynamics of life phenomena*. Philadelphia J. B. Lippincott Co.

Moore, B., and Parker, W. H. 1902. The osmotic properties of colloidal solutions. *Am. J. Physiol.*, **7**, 261.

Overton, E. 1902. Beiträge zur allgemeinen Muskel- und Nervenphysiologie. *Pflüger's Arch. ges. Physiol.*, **92**, 115, 346.

——— 1904. Beiträge zur allgemeinen Muskel- und Nervenphysiol. *Ibid.*, **105**, 176.

Pappenheimer, A. M. 1916. The Golgi apparatus. *Anat. Record*, **11**, 107.

Pauli, W. 1912. Kolloidchemie der Muskelkontraktion. *Kolloidchem. Beihefte*, **3**, 361.

LANE LIBRARY. STANFORD UNIVERSITY

Piersol, G. A. 1920. *Normal histology*. Ed. 12, Philadelphia and London: J. B. Lippincott Company.

Ranvier, L., 1880. *Leçons d'anatomie générale sur le système musculaire*. Recueillies par J. Renaut. Paris: Delahaye et Cie.

Rollett, A. 1885. Untersuchungen über den Bau der quergestreiften Muskelfasern. I. Denkschriften der kaiserlichen Akademie der Wissenschaften. *Wien. Math.-Nat. Classe*, **49**, 81.

——— 1886. Untersuchungen über den Bau der quergestreiften Muskelfasern. II. Denkschriften der kaiserlichen Akademie der Wissenschaften. *Wien. Math.-Nat. Classe*, **51**, 23.

Schafer, E. A. 1891. On the structure of cross-striated muscle. *Internationale Monatsschrift für Anatomie und Physiologie*, **8**, 177.

Schafer, Sir E. A. 1920. *The essentials of histology*. Ed. 11, Philadelphia and New York: Lea and Febiger.

Schultz, P. 1895. Die glatte Musculatur der Wirbelthiere. *Arch. Anat. u. Physiol.;* physiol. Abt.; Jahrgang 1895, 517.

Stengel, A., and Fox, H. 1921. *A text-book of pathology*. Ed. –, Philadelphia & London: W. B. Saunders Co.

Vernon, H. M. 1899. Heat rigor in cold-blooded animals. *J. Physiol.*, **24**, 239.

Section XXIII
CARDIAC MUSCLE

CONTENTS

SECTION XXIII

PAGE

I. GENERAL DESCRIPTION 808
II. CYTOGENESIS 815
III. CYTOLOGY IN RELATION TO BEGINNING OF CONTRACTION 820
IV. CYTOLOGY 821
1. Fibrils 821
2. Transverse striations 822
Z line—M line—Q and J discs—Sarcomere—N and E discs
3. Sarcosomes 827
4. Intercalated discs 828
5. Contraction and contraction band 834
6. Certain physical and chemical changes in contraction 835
7. Sarcolemma 837
8. Sarcoplasm 839
9. Refracting substance 839
10. Summary 840
V. GROWTH 841
VI. SEGMENTATION 842
VII. HYPERTROPHY AND REGENERATION 843
VIII. PATHOLOGY 845
1. Fatty degeneration 845
2. Amyloid degeneration 846
3. Calcification 846
IX. BIBLIOGRAPHY 847

Section XXIII

CARDIAC MUSCLE

A. E. COHN

THE history of the study of histology of heart muscle is synchronous practically with the period during which the cellular nature of living tissue has been understood. More detailed accounts of this history than are possible here are to be found in Holmgren (1910), Heidenhain (1911) and Schafer (1912). A brief note on the development of knowledge of these structures may, however, be given.

Bowman in 1840 was the first to publish an important work on this subject. He saw that muscle was joined together in discs and that there was an end-to-end fibrillary union. In 1849 Dobie gave a fair description both of the z lines and at the same time saw what are now called Q and J discs. He was the first also to mention interfibrillary sarcoplasm. Kölliker (1851) began the study of the relation of fibrils to discs, for this was the subject which occupied contemporary attention most. Following Henle (1841), Kölliker in 1850 and in the years succeeding, was occupied with the question of the existence and arrangement of the granules subsequently to occupy a prominent place in the study of muscle. Krause in 1868 described the z line and laid the foundation for a systematic study of muscle fibrils on the basis of compartments. Merkel (1872) was the next to add a detail to the sum total of knowledge in that like Hensen (1868) he found a line traversing the Q disc. But Merkel's chief contribution lay in his appreciating the fact that, in the course of contraction, the histology of the fiber changes. These changes he associated with movement of anisotropic substance. Engelmann later (1873), in addition to adopting the idea of the partition of fibers into compartments by the z line (intermediate discs or "Zwischenscheiben"), saw that the compartment was made up of layers arranged longitudinally, each having a different refractive power. He likewise studied the changes which take place during the course of contraction (1878).

The next advance was made by Retzius (1881) when following Heitzmann's discovery (1873) he showed clearly the existence of the reticular nature of certain structures of muscle when treated with gold chloride. The view is characteristic of the time, that a contractile theory must depend upon the existence of a reticulum and a contained protoplasm. The significance of Retzius's reticulum is to be found in connection with this theory. It still has a certain vogue, as is to be seen in the work of such investigators as Marcus. The significance of Rollet's work in 1885 and 1886 consists in his recalling attention to the existence of fibrils in muscle, which, according to him, represent the actual contractile substance. Since then, there has been a division among histologists; there are those who regard the fibrils as homogeneous and as supported by a reticulum, the appearance of the cross striation being lent to them by arrangements of the reticulum; those who regard cross striation as a property of the fibrils themselves; and a third group who, like the first, believe the muscle fibrils to be homogeneous and see in the existence of granules closely applied to the fibrils the occasion for the cross-striated appearance. Among those who have been concerned in recent times with the subject of the granules is especially to be mentioned Holmgren (1907 and 1910). As to the reticulum, authors differ on its precise nature. Some, like MacDougall (1897), think that the fibrils are traversed by the membranes at the levels of the z and M lines, while others, like Marcus

(1925), view these lines as bands which surround the proper fibrillary substance. On the basis of these different views of the structure of the muscle depend theories of the mechanism of contraction with which they are more or less closely correlated. A detailed description of these theories is not given in this paper. An account of them is to be found in v. Fürth (1912).

On account of the difficulty of analyzing human striated muscle, both skeletal and cardiac, the effort has continuously been made to obtain light on its structure by having recourse to the study of muscle in other species. Arthropods and amphibians have especially been selected for these investigations, but even here the difficulties are great. In some respects they are insuperable. How closely what has been found in other species is characteristic of the state of human muscle frequently cannot be stated. The transfer in any case is always difficult and may be impossible. The comparative method has the same advantage here as elsewhere. To attempt to furnish here all the evidence which has been accumulated would lead too far and would increase the bulk of the following description inordinately. This report is therefore confined more or less narrowly to the study of human heart muscle.

This discussion of the cytology of heart muscle begins with an arbitrary statement of the structure of healthy adult muscle in order to introduce the subject matter quickly and to avoid description of details and of variant theories. A report of these matters is postponed until they can be analyzed under their several heads.

I. GENERAL DESCRIPTION

It is now generally admitted that the heart muscle, which resembles skeletal muscle in general in being striated transversely, forms a syncytium; that is to say, a structure in which there is branching and a reuniting of the fibers. The fibers are surrounded by a membrane called the sarcolemma. Within the fibers or trabeculae there are nuclei which have a slightly oval form and which are placed on transverse sections near the center of the fibers. The intervals are close and seem to maintain a degree of regularity. In addition to the nuclei there are contained within the sarcolemma the proper substance of heart muscle; namely, the fibrils. Surrounding the fibrils, which have presumably a certain degree of consistency, and of course within the sarcolemma, there is the sarcoplasm, the more fluid constituent of this anatomical system. The fibrils are elongated structures, the exact diameter of which it is impossible to give for reasons which will appear shortly. Their length is likewise unknown for it is impossible to follow them throughout their extent. It is indeed not clear what the nature of their ends may be, for, within the structure of the mass of the muscle, these have not been seen except perhaps in teased preparations. Presumably, therefore, the fibrils pass through a succession of trabeculae and their anastomoses. An idea of how this continuity is attained may be gained from observation of Purkinje tissue in which there is the possibility of tracing the passage of individual fibers through a number of adjacent cells (Section XXIV). The fibrils themselves present an intricate structure. They lie parallel to one

another and tend, on longitudinal section, to present a uniform, regimented appearance which depends upon the fact that they are divided by transverse lines in such a way as to give the impression of being composed of discs of approximately equal height piled one on another. There is no portion of the structure of the muscle which has not given rise to detailed investigation and to controversy; indeed, the actual arrangement of the structure and meaning of the transverse striations have been, and still are, the occasion for vigorous research and discussion.

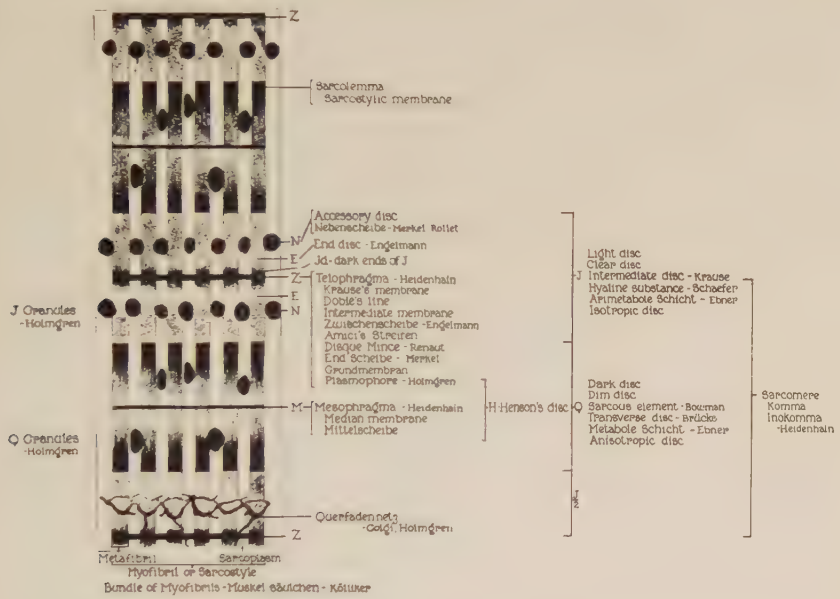


FIG. 354.—This figure is taken from M. Heidenhain (1911). It contains as many of the synonyms of the various structures in heart muscle fibrils as seem current at the present time.

It is necessary to divide the description whether of the fiber or fibril, into the uncontracted and the contracted phases. In the *uncontracted* phase there are to be recognized so-called light and dark discs (Fig. 354). In this state the dark disc usually referred to as the Q disc is composed among other substances of anisotropic, that is to say, double refracting substance. Beyond this disc lies the light disc usually called J, within which is contained either none or, relatively speaking, a small amount of double refracting substance. The J disc is bisected by a thin disc or line called Z, on each side of which lie the end discs, E, which are separated from the J discs by the accessory discs, N. A fibril, then, or a group of fibrils forming a fiber, presents a succession of dark and light discs, each with an appropriate and complex structure.

The unit of a fibril has been called a sarcomere and lies between two z lines or discs called telophragmata (Heidenhain). In the diagram (Fig. 354), to avoid confusion, the synonyms for this and for the other structures are

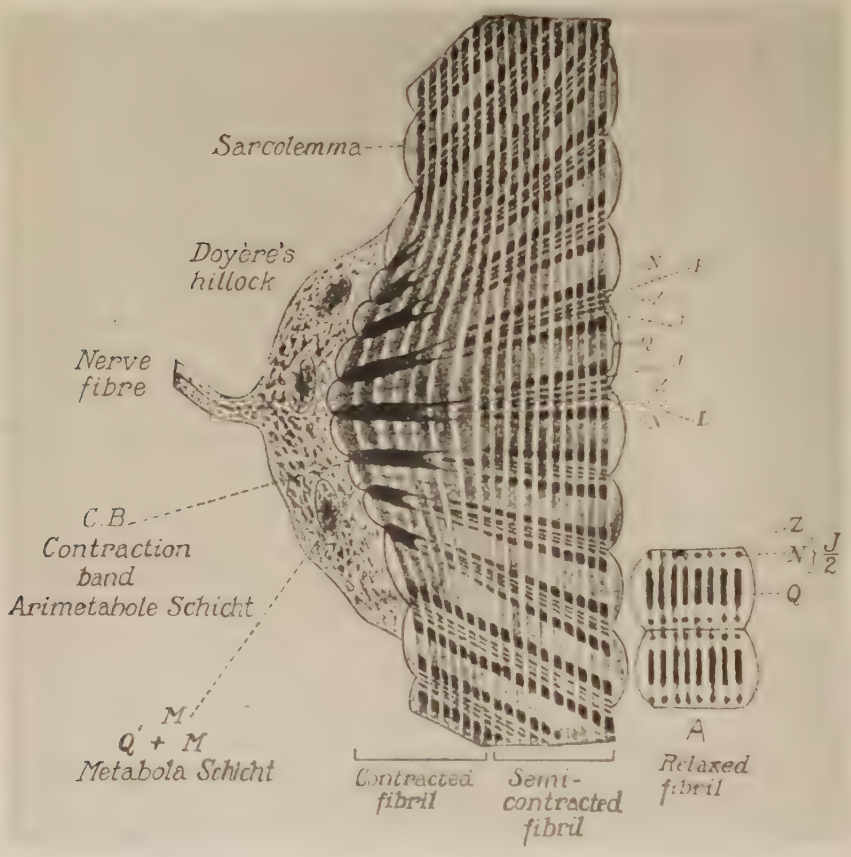


FIG. 355.—This figure is reproduced from H. E. Jordan (1920). It shows at the left a lateral "contraction wave" in which eight contraction bands are seen (according to Rollet). At A there are two sarcomeres which illustrate Jordan's conception of the histology of relaxed muscle. At the right of Rollet's figure the mid-phase of contraction (according to Jordan) is represented. At the left the broad dark bands are the contraction bands. These include halves of two successive Q discs and the included N, E and J discs. In the center is the z line. The contracted state of the muscle represents a reversal of striation so far as staining substance of Q discs and z lines is concerned, Q is now becoming light and being bisected by M, and z becoming a contraction band composed in the manner already indicated.

given. A sarcomere may, then, be described as beginning with a z disc to which an end disc E is adjacent, followed by an accessory disc N, beyond which is found the major portion of half of the light J disc, then the Q disc,

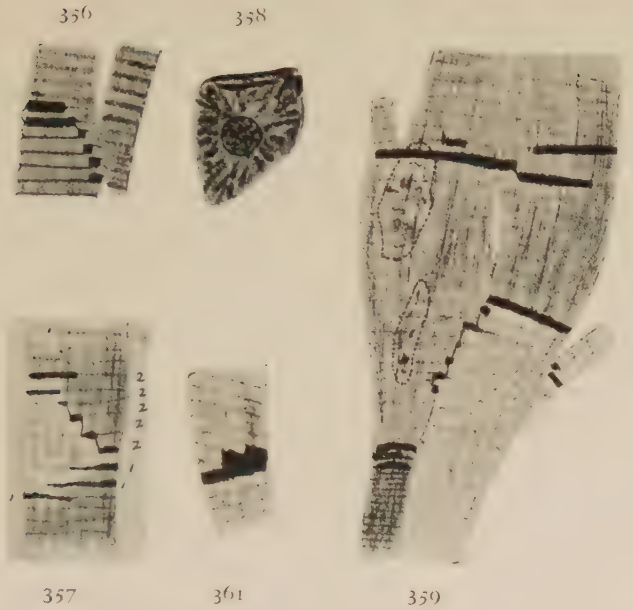


Fig. 356.—Represents a branching trabecula from the right ventricle and shows a terraced form of intercalated disc. Above the disc the muscle is relaxed, below it is contracted. In the relaxed portion z lines, q and j discs are conspicuous. The sarcolemma is festooned and connects the z lines. At the intercalated disc the sarcolemma joins its mid-portion. In the lower four segments the upper limits are on a level with the z lines to the right; the lower limits correspond to the contraction bands at the left. (Reproduced from Jordan and Banks, 1917.)

Fig. 357.—According to Jordan the wedge-shaped lighter portion at the left represents relaxed muscle. The discs marked 1 shade into z lines, those marked 2 are parts of a terraced disc. The origin of discs like this is complicated. Its form according to Jordan is dependent upon fusion of originally separate parts of the cardiac syncytium. When the disc is oblique to the assumed line of fusion it is difficult to say. It may be due to new mechanical conditions under which the fiber has come to exist. (Reproduced from Jordan and Banks, 1917.)

Fig. 358.—A transverse section of a fiber is shown. The intercalated discs are superficial and do not, at least at the level of the section, pass through the whole fiber. (Reproduced from Jordan and Banks, 1917.)

Fig. 359.—This figure shows adjacent trabeculae from the right ventricle. In the upper portion occur four simple band discs. The upper one on the left extends across the nucleus and on both sides into adjacent trabeculae. Below and to the left there are three discs at the levels of three successive z lines. In the center, where the two main trabeculae fuse, a step-like disc is seen. The risers here according to Jordan represent fused sarcolemmae. (Reproduced from Jordan and Banks, 1917.)

Fig. 361.—Intercalated discs like the one shown in this figure are not infrequent. At present it is difficult to interpret. Both histogenesis and mechanical necessity may have shares in its development. (Reproduced from Jordan and Banks, 1917.)

again another half of the major portion of the J disc and finally N, E and Z discs.

In addition to nucleus, discs and sarcoplasm, the muscle fiber contains an elaborate apparatus of granules. These are arranged predominantly in two places; first, the Q granules, within the Q disc, and second, the J granules close to the Z line. The granules, arranged as it were in strings, are found between the fibrils, the fibril being more or less completely surrounded by strings of this nature as if they were palisades. Additional infrequent ones occupy small portions of the sarcoplasm.

In the *contracted* state (Fig. 355) the muscle undergoes what has been called a reversal of striation. In this state the Q disc becomes elongated and

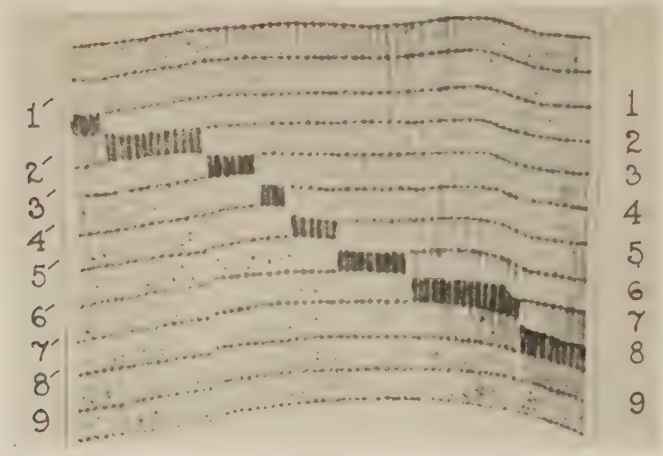


FIG. 360.—This figure is taken from M. Heidenhain (1911). It shows a terraced intercalated disc the upper borders (1–9) of the successive steps of which to the right are Z lines. The lower borders (2–3) are not the Z lines of the next sarcomeres but are Z lines which are apparently dislocated. The total number of Z lines both to the right and to the left is the same, 9.

paler, the paling taking place at its mid-level, the ends of the discs taking on a somewhat darker color. The faded portion in the middle is called Hensen's disc and this is not infrequently bisected by a fine line M, called also the median membrane or "Mittelscheibe." The Z line is also transformed and takes to itself the neighboring two E and N discs, and presumably the J granules. A completely contracted fiber presents, therefore, two discs, a dark one and a light one, the light one being all that is left of Q now become the H disc, and a dark one Z, now composed of all the discs into which the J disc is divided in the uncontracted state plus, as some think, those portions of the Q disc which have spread apart as the H disc forms and which have migrated to the neighboring Z line. The transformed Z line is now called the "Contraction Band."

So far, in most details of the histology of the fibrils, heart muscle resembles the structure of skeletal muscle. It differs, however, from ordinary striated muscle in two striking particulars. First, there is its syncytial arrangement; but aside from this, heart muscle presents another characteristic structure, the intercalated disc. These are found at levels where z lines are expected. They may be seen sometimes with, sometimes without appropriate stains at irregular intervals along the course of the fibers. Their form takes on a great variety (Figs. 356 to 365). They are often spaced at intervals of 20 to 40 or more sarcomeres but sometimes they are found close together, separated by not more than one, two or three discs. Usually in healthy muscle, they are straight and run across the entire diameter of the fiber, but others terminate after stretching across not more than a quarter the distance. Besides the straight ones, intercalated discs are often found which have a step-like arrangement, the steps going not in one direction only, but after having begun in one, turn to assume an opposite direction. The term "step-like" accurately describes their appearance, for a riser passes over into of a tread; there is usually no super-position of two horizontal segments. The height of the discs is not always uniform; they appear sometimes to maintain the same dimension half-way across a fiber and then come gradually to a point. Occasionally a disc at each of its two extremities gives the impression of continuing a short oblique course upwards at one end, at an optical level different from that of the central portion, and downwards similarly at the other. Such discs give the appearance of being short spirals (Fig. 362).

The description so far given is a brief statement only of the histological facts relating to heart muscle and is given with reference to no particular time in its life history. Obviously heart muscle does not have the appearance which has been described either in the embryological period or in the period of advanced years. It does, moreover, take on characteristic changes in the course of disease. It may be that certain features which have already been mentioned, such, for instance, as the more bizarre forms of the intercalated discs, are not natural but do in fact occur rather in connection with alterations which take place during hypertrophy of the heart muscle.

Of the relation of age to the form of the fibers, there is little of specific reference in the literature of this subject. This point will appear in a striking fashion in connection with the occurrence and number of the intercalated discs. It will appear also in connection with the height of the Q and J discs, with alterations in the form of the nuclei and with the development of perinuclear pigment. Changes in all these respects apparently take place with time and are appropriate to the anatomy and physiology of different age levels. It will also be interesting to notice how slight has been the definitive correlation between the function of contraction, that is to say, of longitudinal shortening of the muscle, with specific structural elements that have been named in this description of heart muscle. In the analysis of

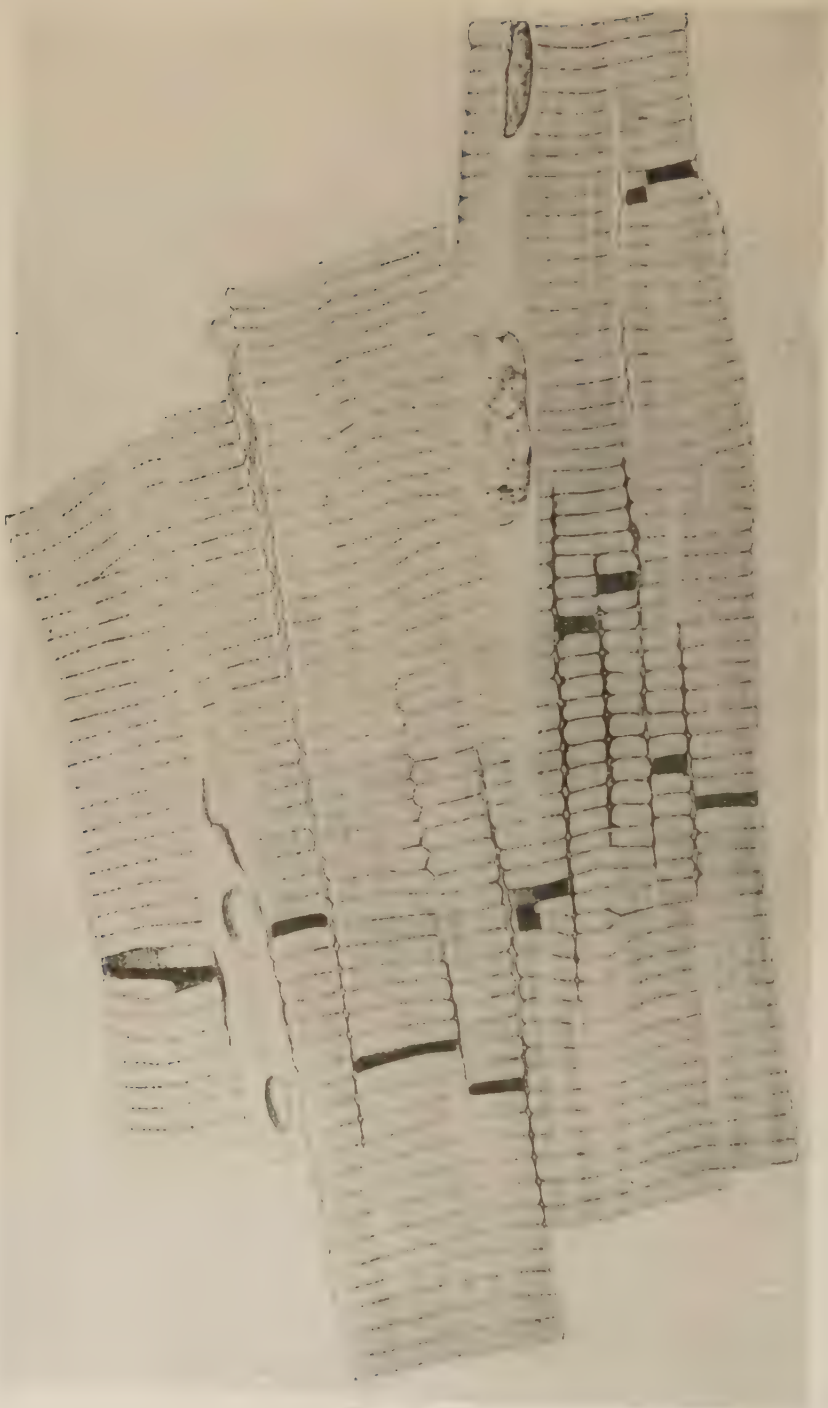


Fig. 362.

the relations which exist between these elements and the mechanism of nutrition, scarcely a beginning has been made; while of respiration and energy exchange, although through the work of Hopkins, Fletcher, Hill, Meyerhof and Parnas exact knowledge exists, there is little to associate this knowledge with actual histology. An understanding of the alterations which take place in disease is still less developed and in all probability cannot be more clearly defined until normal alterations in structure and in function of contraction are brought into closer correlation. Rational attempts at interference (therapeutics) with natural processes like that of contraction, are probably inevitably postponed until greater insight into the mechanism of these processes is attained. Although valuable information is available in several directions, the state of knowledge is still rudimentary.

II. CYTOGENESIS

Of the genesis of human muscle there is very little direct information. There has been controversy as to the precise method of origin of the contractile elements.

There are those who regard their origin as taking place as the result of a union or coalescence of myoblasts. The fibrils are said to arise from small structures within the cells called chondrioconts, chondriosomes or mitochondria (Godlewski, 1902; Duesburg, 1909; and Asai, 1914). There are others (Heidenhain, 1911; Iwanaga, 1925), however, who regard the origin of fibers as being developments from single rather than multiple myoblasts. The smallest embryos in which the fibrils are found are 5.8 cm., though here they are sparse (Iwanaga, 1925). Growth does not take place by the coalescence of myoblasts; on the contrary, fibers develop always from single myoblasts. Development from young cells which lie between older ones may later take place. According to Iwanaga, the number of fibrils increases and they are found, in cross section, distributed around the periphery of the fiber. On the multiplication of the fibrils they give to the structure at the age of 9 cm. the appearance of a thick-walled hollow cylinder. At first they are not uniformly distributed but congregate in clumps and seem to push the nucleus from its central position. The nuclei are elongated structures which run parallel with the long axis. They divide usually by mitosis, the new nuclei taking up their positions in a longitudinal row. In the long axis the nuclei are oval. On transverse section they are rhombic and triangular at the age of 10.5 cm. and then kidney-shaped at the age of 13.0 cm., the convex surface being placed towards the circumference of the fiber. At the 15.5 cm. stage most of the nuclei are to be found at the surface, at 25.0 cm. most of them seem to have wandered outside the fiber so that only a few remain within it. At the 33.0 cm. stage the change in position is complete. According to Iwanaga, it is the pressure exercised by the multiplication of fibrils which determines the change in position of the

---

FIG. 362.—This figure is taken from M. Heidenhain (1911, Fig. 316). There are to be seen examples of what Heidenhain calls "Zwischensarkolemmae;" in the center fiber an example of a Vernier arrangement of the fibrils in which the zigzagging of the sarcolemma and its attachment to the z line of both adjacent fibrils is seen; and in the right fiber an intercalated disc which appears to have a spiral form, the spiral being superficial as is indicated by the different levels at which it appears, the ends of the spiral being deeper.

nuclei. It should be understood that this description refers to the development of skeletal muscle in human fetuses. After the fibrils have developed so far by multiplication, there appears to be, at least in the extremities, a stage of degeneration, sarcolysis (Schafer, quoted by Iwanaga). Forms which could be described as evidence of regressive changes have been observed by several investigators, but whether these are actually representative of the developing process in the sense of being a complete destruction of muscle seems to be open to doubt. It may be that the cytoplasm disintegrates, leaving the nuclei almost bare. About these, then, there develops a new protoplasm which is at once fibrillary in structure and in this respect unlike the original fibers. At this stage mitosis is not seen. The process here described as sarcolysis seems to be peculiar to the develop-

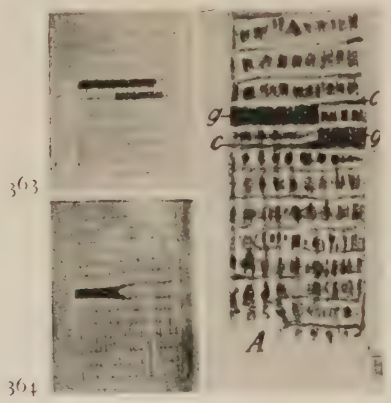


FIG. 363.—This figure shows two intercalated discs within the same sarcomere. (Jordan and Banks, 1917.)

FIG. 364.—In this figure from the atrium there is shown an intercalated disc which bifurcates, the ends passing to two successive z lines. Jordan reproduced it as being the kind of disc which might support the idea that sarcomeres develop from inte calated discs. Jordan's interpretation of the figure is that it may represent "partial failure of reversion in opposed halves of two successive contraction bands." (Jordan and Banks, 1917.)

FIG. 365.—This figure represents a curious arrangement in which the intercalated discs G apparently pass into the contraction bands C, which in this case represent Z lines whereas the main portions of the discs pass into Q. (v. Ebner, 1920.)

ment of muscle in the extremities but not necessary to that elsewhere in the body, as, for instance, in the tongue. A process similar to sarcolysis in the muscle of a human extremity has not apparently so far been described as occurring in heart muscle. It is a striking fact that sarcolysis is complete before motor nerve ends become associated with the muscle. There are, then, two methods of growth; that in which sarcolysis is involved (extremities) and that in which new fibers are continuously developed, but no coalescence of them.

According to MacCallum (1897) who studied the origin of heart muscle fibers in pigs there appear on cross section at the age of 10 mm. spindle-shaped cells composed of nuclei and fibrils which are divided into discs. In some cells, he says, the fibrils show adult striation. The tissue is composed at this stage (10 mm.) of four types of cells. There are some in which there is an irregular network, in the meshes of which there is a homogeneous substance. There are others with more regular network. Then there are those in which the network is subdivided and finally those in which in each mesh, at

the periphery of the fiber and at the junction of sarcoplasmic disc, fibril bundles appear each apparently surrounded (Fig. 5, MacCallum) by sarcoplasm. Sarcoplasmic discs are equivalent, it appears, to sarcomeres (Fig. 354). MacCallum describes five developmental stages, represented by the four types of cells found at the 10 mm. stage plus the adult cell. At the 13 to 15 mm. stage there are still only occasional fibrils scattered

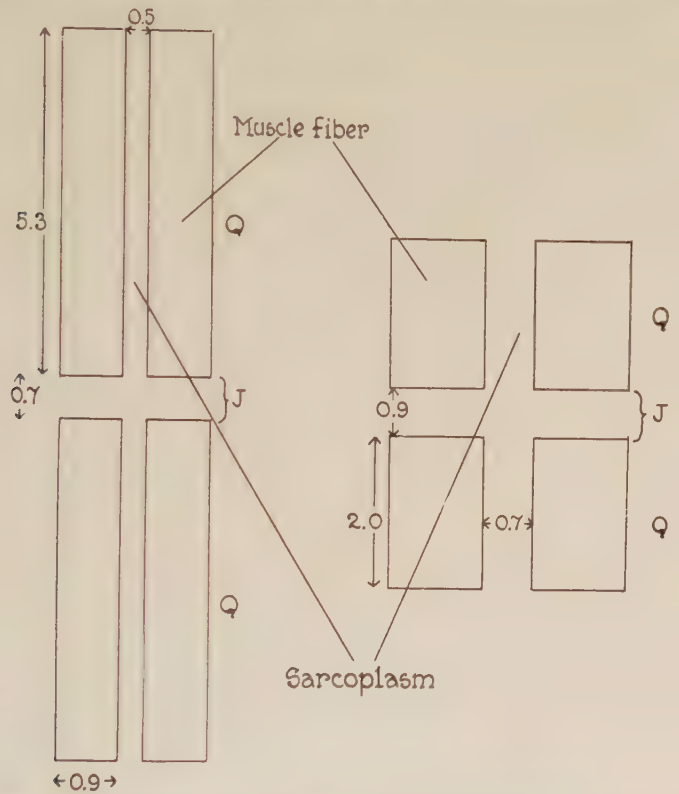


FIG. 366. —These measurements of Hydrophilus are taken from Hürthle (1909) They are reproduced in the form of a diagram by v. Fürth (1919). The measurements of thickness at rest are taken from Hürthle, Table 3; thickness during contraction from Table —; and measurements of height during contraction and rest from Table 5. It appears that contraction results in no alteration in volume of the muscle; a minimal change takes place in the height of I; and a fairly marked change in Q so that Q becomes shorter and wider. Q (rods plus sarcoplasm) apparently decreases 15 per cent. Since the rods remain unchanged, it is presumably at the cost of the sarcoplasm that the decrease takes place. In view of the fact that J increases slightly sarcoplasm may pass from Q to J.

through the cells. By the time the 20 mm. stage is reached most of the cells contain them and at the 35 mm. stage all the cells do. At 55 mm., when the cells are elongate, they take on the appearance, in cross section, of the adult fibril. At 72 mm. the general form is adult. The developed cells are to be found predominantly at the periphery of the heart muscle mass, the less developed towards the interior. Mitoses are everywhere to be found, but are more numerous towards the inner side. It will be seen that in

MacCallum's view there is development of a mesh, first coarse and then, as the result of subdivision, fine, and that at the points of intersection of the network, fibrils develop. Fibrils, then, are concentrations of tissue growing out of the network. The actual structure of the fibers is made up of units of which sarcoplasmic discs are the base. The sarcoplasmic discs are completely surrounded by the network, the z lines being continuous with the network membranes.

Witte (1919) corroborates this description of MacCallum's. In embryos of 25 mm., she says, spindle cells appear. At 38 mm. cross striations are to be seen at the periphery and at 76 mm. there are no longer spindle cells but a definitely developed striated syncytium. The syncytium arises by the union of spindle cells. At the points of union there are lines of demarcation, and these are the protoplasmic bridges between cells.

In beef hearts, Jordan and Banks (1917) describe a process similar to that seen by MacCallum in pigs. At three months the ventricular muscle passes through a stage in which the cellular elements closely resemble smooth muscle. The cells are close packed, slender and fusiform. In an earlier stage cardiac muscle as well as smooth muscle is made up of stellate and irregular cells which anastomose to form a syncytium. Apparently smooth muscle, cardiac and striped skeletal muscle all pass through similar stages in the course of development.

A somewhat different account of the origin of fibers is given by Meves (1907–1909) and Duesberg (1909) in the case of the development of skeletal muscle in chicken embryos. In this account growth does not take place by longitudinal splitting but by development of fibers after a manner which is a repetition of the way in which the original fibers develop. The cytoplasm of all cells, including muscle cells, contains mitochondria. The first fibers develop through coalescence of neighboring cells. Innumerable undeveloped cells remain lying between them, for the most part spindle-formed. These fibers become cylindrical; fibrils develop within them from mitochondria. The fibrils then come to lie in the periphery of the cells, the nuclei in the interior. The process, so far as the individual cell is concerned, apparently stops. Other spindle cells which remained between the fibers and which have not yet developed fibrils now attach themselves to the latter in such a way as to form a coat. The coat sometimes encloses a single fiber, sometimes a number. These coating cells are myoblasts. They arrange themselves in rows and themselves develop fibers in which fibrils again arise.

The fibers in the heart, according to Godlewski (1902) who proposes a different theory, develop granules which form into rows first and then fuse into homogeneous fibrils which in turn become striated. These changes take place while the tissue is still in the mesenchymal stage. At the time when the fibrils are formed they are contained in irregular territories, that is to say, within cells. The cells or cell territories coalesce, the fibrils within them at that time extend and pass several nuclei on their way. At first the course of the fibrils is irregular, but later it straightens. They increase by longitudinal division.

Marceau (1904) gives still a different account. In his description fibrils appear first, then differentiate into granules which are primarily single and later form pairs. The paired granules are equivalent to what becomes in the adult a single Q disc.

In Heidenhain's (1911) view heart muscle fibrils are laid down as ultra-microscopic elements (metafibrils) which he calls protomeres. They gradually increase in size (diameter) until they attain microscopic visibility and are called myofibrils, having a transverse dimension of 0.2μ. Groups of myofibrils form muscle bundles (Säulchen) and these in turn form fibers. They increase by division at the myofibril stage. Of the mechanism of the origin of metafibrils, Heidenhain proposes no theory. The original threads from which protomeres develop may, in his view, as in that of Meves, have been present in the fertilized ovum. Subsequent increase in number is due, according to Heidenhain, to

longitudinal division. Whether the multiplication of fibers takes place by the development within each fiber of an increasing number of fibrils until the fiber is ripe for division, or whether the fibers divide first and fibrils subsequently develop within them is unknown. The multiplication of fibers provides for what might be called lateral growth.

In addition to lateral growth the problem of longitudinal growth requires consideration. The segmented structure of heart muscle appears to give a clue to the manner in which growth in this plane may occur. The unit at the basis of the longitudinal system of striated muscle is the sarcomere, called in this connection by Heidenhain, the histomere. Heidenhain fortifies his belief in growth by the division of histomeres by referring to the method of growth in general, whether of persons, organs, cells, cell portions or protomeres. The sarcomere, it will be remembered, is included within two succeeding z lines. If longitudinal growth occurs, the method should be established by discovering evidence of the division of histomeres. Evidence for growth of this kind Heidenhain finds in sections of Triton larvae. At the ends of certain fibers he finds shadow-like structures in which there can be detected no histological differentiation. It is here that differentiation must, he assumes, take place. He cites the example of the notochord which is at first more or less homogeneous but subsequently is converted into the complex structure of the vertebral column. The method of growth in these homogeneous fibril ends must take place by assimilation, increase in size and by subsequent division. To account for growth in the heart on this basis the process can take place only at the ends of fibers, presumably only in the papillary muscles or at the arteriovenous rings. No other location suggests itself on the basis of what is now known of the anatomy of the heart. But if growth were to take place only at these sites, distortion of the heart would in all probability ensue. This method of growth must therefore be abandoned unless v. Ebner's (1920) finding of loose pointed ends of heart muscle fibers within the mass of the cardiac muscle may be taken as evidence in support of Heidenhain's view as points other than those just mentioned in Triton larvae in which progressive differentiation and growth may take place. In the absence of knowledge of fibers with free ends Heidenhain turned to intercalated discs with the idea that these might be interpreted as centers at which growth is initiated. Marceau varied this notion of Heidenhain's to the extent of thinking that it was not the intercalated discs themselves but the adjacent discs which formed the growth zones. In a certain sense Heidenhain was willing to agree with Marceau on this point for he came to believe that the intercalated discs are analagous to intercellular substance. Whether intercalated discs are to be regarded as growth centers is a matter of considerable importance. At all events it is to the credit of Heidenhain that he was the first to point out the need of finding in heart muscle a technique for longitudinal growth. Additional objections regarding intercalated discs as growth centers have been suggested. One of the chief objections to this interpretation is the fact that both according to Heidenhain (1902) and to Aschoff (1906) they are lacking in fetal hearts and are indeed first seen in the hearts of children. Dietrich (1906) has also confirmed this fact. It was pointed out, furthermore, by Cohn (1909) that the number of intercalated discs increases progressively from birth to old age. Jordan and Banks (1917) allude to the fact that they do not disappear but actually increase at the end of the growth period of the organism. They point out also that they do not look like fetal sarcomeres.

From all this it appears that both the method of lateral and of longitudinal growth require further investigation. It appears to be desirable, moreover, that studies in growth be continued not only at that stage of development after trabeculae, fibrils and their segmental structure are established but also in the fetal period at the time when the cardiac syncytium is in the stage of formation.

III. CYTOLOGY IN RELATION TO THE BEGINNING OF CONTRACTION

On the subject of the structure of heart muscle at the time when contraction actually begins, there is as yet little information. It has been found that the heart begins to beat before fibrils are visible in chickens by Eckhard (1866) and Kurkiewicz (1909) and in the blindworm by Marceau (1903). Beside giving these references Heidenhain mentions having seen both homogeneous and striated fibrils in the duck embryo three days old (1899). But the chief evidence so far is derived from observations of isolated hearts and of fragments from them in tissue culture. Burrows (1912) was the first to show that isolated cardiac cells could beat under these conditions and that each could do so independently. Lake (1916) and Shipley (1916) confirmed these observations. M. R. Lewis (1920) says that at the time contraction begins, no characteristic structure is to be found in the cells but that the tissue exhibits a high degree of refraction. There are no myofibrils. There is no transfer of substance from granule to myofibril for there is no change in the granules and there are no fibrils. In M. R. Lewis's opinion contraction in these cells, living in Locke-Lewis's solution, takes place when they are single, slightly differentiated structures. If there is a criticism to be made of these observations it is that sufficient evidence on the presence or absence of myofibrils is not adduced. At the time when contraction begins, that is to say at about the 10 myotome stage, at the twenty-eighth to thirtieth hour of incubation, cross striation is difficult to find. It is present but it is detected only after careful search at the period between the 11 myotome stage and the second day (M. R. Lewis, 1919). Cross striations are found not so well in living as in fixed material. At the time when the fibrils appear these are cross-striated.

Fibrils can be produced apparently at will, "showing that the form assumed is due to factors present during the fixation of some substance in muscle tissue." It is perhaps true, however, that if during the period of fixation, structures which are termed fibrils appear and do so in a uniform fashion, there must exist in the living tissue substances in some definite form and arrangement which yield the appearance of fibrils in the fixed state. There is nothing in the second half of the statement which has been quoted which is at variance with this view. It may be conceded, therefore, that whatever value for the understanding of heart muscle fibrils have, their detection, even under these conditions, for the purposes of classification and of other studies is significant. Additional information on the relation of structure to function is given by W. H. Lewis (1923). In his study, individual cells were seen to pulsate in tissue culture. These cells migrated and formed a reticulum but not a syncytium. Myofibrils did not appear in the living cells although they did so on fixation. Under these circumstances myofibrils vary in thickness down to the limits of visibility, as was pointed out by Heidenhain. In the fixed state some of the cells showed fibrils which were

cross-striated. In others the fibrils were smooth and in others still none was to be seen. It is important to notice that whether they were cross-striated or not, all these cells beat rhythmically. The fibrils were not seen to extend from cell to cell. In living animals, both embryo and adult, fibrils are probably not to be found. It is likewise stated that living heart muscle is probably composed of individual cells without fibrils.

There is another structure in the living heart of chicken embryos forty-eight to seventy-two hours old. It consists, according to Davis (1924), of a jelly-like substance between the myocardium and the endocardium. This substance is, apparently, entirely structureless and does not escape from the heart on section. It must, therefore, be closely adherent both to the myocardium and endocardium. The fact of its existence and the nature of its structure are confirmed in stained sections and in dark field illumination. Its significance seems to be unknown. According to Olivo (1925), contraction begins at the 9 somite stage, exceptionally at the 7 somite stage. When contraction begins there is a myoepicardial sheet which looks like epithelium and is composed of a single or double membrane of cubic or prismatic cells. These are closely connected. There is no trace of myofibrils. The first contractions appear to be purely sarcoplasmic. Sarcoplasmic contractions continue and, when they appear, are synchronous with those of the myofibrils. The myoblasts are uninuclear. In them the myofibrils appear first smooth and then striated. But the myotomes, he says, are irritable to electrical stimulation before the development of myofibrils. It appears, then, that activity may begin before the differentiation of these elementary muscular structures.

IV. CYTOLOGY

1. *Fibrils:*

The fibrils, according to Heidenhain (1911), are to be defined as the smallest indivisible elements of heart muscle when these are imagined to be split longitudinally. How small the diameter of a fibril may be, varies. In *Hydrophilus*, Hürthle (1909) obtained a figure as small as 0.87μ. Others place them as being 1μ and 2μ. Fibrils of this nature may be found both in the living and the fixed muscle. Measurements as small as 0.2μ have been obtained. Heidenhain points out, however, that this dimension is at the limit of visibility; that is to say, is just within the power of the microscope to resolve. Heidenhain calculates that there are between 175 and 210 million fibrils per square centimeter. With these figures those of Karsner, Saphir and Todd (1925) may be compared. They found 7700 million per 100 c.c. or about 383 million per square centimeter. It has been said before that in Heidenhain's view the fibril is the development of an ultra- or meta-microscopic structure. Between the meta-microscopic and the microscopic fibril there is no sharp division. Fibrils are metafibrils when they have attained

the stage of visibility (Fig. 354). Metafibrils belong to the order of protomeres. The smallest muscle unit that can be identified then has as its transverse dimension a magnitude not smaller than 0.2μ to 0.3μ. Usually fibrils are given as being larger than this, that for *Hydrophilus* by Hürthle as already mentioned being 0.87μ. Human fibers may attain dimensions between 1μ and 2μ to 4μ.

The histological unit is, then, apparently a myofibril which has no definite or specific dimensions but is made of an indefinite number of metafibrils. There is, of course, implied in this that the dimension of a structural unit is determined by the resolving power of the microscope and is placed as 0.2μ because this figure represents the limit of visibility. The structure called "Säulchen" is a union of a number of fibrils. Fibrils, according to all these investigators, are differentiated structures, presenting the familiar striated appearance.

Marcus (1925) takes a different view of the structure of a fibril. He regards this element as a hollow tube filled with unstained, structureless fluid, the sarcoplasm, surrounded by myofibrils and by a darker and denser shell consisting of longitudinal bands secured by transverse circular bands. The longitudinal bands he names "tonofibrils." They are a supporting framework and have no contractile function. Of them a fibril has from four to seven. These are fastened to the transverse bands which coincide with the well-known Z and M lines. The points of intersection are prominent knots. To the myofibril he assigns a dimension of 1μ. Within a myofibril there is a longitudinal central light strand. The segmental description of the discs does not differ materially from that conventionally given except that the Q discs are hollow, resembling doughnuts. In comparison with Heidenhain's myofibril Marcus's is not at the limit of visibility but is a stocky structure. Transverse striation results from the recurrence of the knots. The fibrils themselves being structureless do not share in the formation of transverse striation especially in the resting stage. The Q disc at this time is due to the application of Q granules (sarcosomes) to the fibrils at this level. The Z lines, or in the stage of contraction, the contracting bands (or C bands) depend on the droplets or granules there accumulated. The question may be raised as to whether confusion in distinguishing myofibrils from tonofibrils may not occur. To this difficulty Marcus's answer is that myofibrils may be distinguished by a difference in diameter, the absence of transverse striations and a different relation to transverse lines.

2. *Transverse striations:*

Heidenhain (1911) has distinguished two classes of transverse discs (Fig. 354). In the first class are the lines Z and M, called by him "inophragmata." The Z line bisects the I disc; the M line, the Q disc at the stage of contraction. Depending upon the stage of differentiation in the staining

process, these lines appear to be either homogeneous or composed of rods placed parallel to each other and to the long axis of the fibrils. These lines (Z and M) have architectural significance and have nothing to do with the contractile substance of the tissue.

(*a*) Z LINE

The Z lines or discs bound the sarcomere on both sides (Fig. 354). This disc, Z, is said to measure 0.2 μ. In the uncontracted state Z is double refracting (v. Ebner, 1920). In weak acids these discs in the skeletal muscle of vertebrates can be isolated by the solution of Q and the rest of the J substance. It is perhaps interesting to notice that, in photomicrographs taken of auricular muscle of living frogs during diastole, Z lines were not to be seen (Hürthle and Wachholder, 1922). On account of the rapidity of the motion and on account of the technical difficulties connected with this fact, no photographs have yet been taken during systole. The Z line stretches easily, having considerable elasticity. But it also has a certain amount of stiffness, a characteristic which is observed when the muscle is swollen, for then it does not yield but gives the fibril a beaded appearance. It is firmly bound to the sarcolemma, passing from one fibril across the sarcoplasm to the neighboring fibril where it is continuous with its Z disc at the same level. The Z line, moreover, does not shrink when the muscle is dehydrated, because its greater stiffness maintains it rigid. This is likewise the level at which, as will subsequently be pointed out, segmentation of the muscle takes place. The rods of which the Z line is composed are continuous with the myofibrils which pass from segment to segment of the fibrils. Its structure presents a difficulty: if it is membranous the myofibrils must find a way of passing through it, or conversely it must find a way of passing through them. A way out of this dilemma is Marcus's (1925) plan, according to which the Z line or disc splits and passes around the fibrils like a ring or band. At the far side the halves of the ring reunite and pass on through the sarcoplasm to the next fibril, when a repetition takes place. The continuous passage through fiber and sarcoplasm is a point maintained both by Heidenhain and by Jordan, but is denied by v. Ebner (1920).

Additional evidence for the fact that Z passes from fibril to fibril across intervening sarcoplasm is Jordan's (1920) statement that a Z line can be seen to bend in the intersarcostylic space between two fibrils. This fact is more difficult to demonstrate in the case of M, a structure which is admittedly more fragile and more easily broken by histological fixation. Under these circumstances it becomes necessary to assume that when sarcomeres are found at different levels, as they sometimes are, these delicate transverse connections must be fractured.

(*b*) M LINE

The second disc of the first class, M, bisects the Q disc. It appears, however, only under certain conditions and is especially delicate. The time of its appearance is during the contractile state. During this process there appears, beginning at the middle of the Q disc, a rarefaction, so that a light band comes to lie between the separating halves of the Q disc. It is in the middle of this light band that M appears. The M line is much finer than Z, fibrils pass through it, or perhaps at this level condensations or thickenings appear along the course of the fibrils; that is to say, it is granular. According to Heidenhain (1911) and Marcus (1925), it is attached to the sarcolemma, though this fact is difficult to demonstrate. Like the Z line it passes through the sarcoplasm between adjacent fibrils and is continuous with that of the next fibrils. So far as measurement is concerned, this disc is also stated to measure 0.2μ, although it is manifestly smaller than Z. Accuracy is difficult, for again one is dealing apparently with the limit of visibility of the microscope. The M line is to be seen, then, only in the stage of contraction. When the muscle is in the resting state, Q is homogeneous and H (and consequently M) is not visible (Jordan, 1920; v. Ebner, 1920).

It may be said in summary that the cross striations of the first class are the inophragmata Z and M of Heidenhain. Z is called by him telophragma and M, mesophragma. Two telophragmata, together with the sarcolemma joining the two ends of a pair, constitute a kind of network within which the sarcomere is included. This is, to all intents and purposes, the same unit which MacCallum (1897) describes as the sarcoplasmic disc. It is within the borders of this structure that fibrillary differentiation takes place. Whether this arrangement of telophragmata and of sarcolemma actually forms a network of the sort suggested by Heidenhain is a matter open for discussion. Marcus (1925), as has been said, regards the Z lines as rings or bands and does not believe that they represent discs or plates which penetrate the fibril.

(*c*) Q AND J DISCS

In a second class of cross striations Heidenhain includes the Q and J discs. These differ from the inophragmata in that Z and M are regarded as architectural structures, whereas Q and J are contractile elements. They (Z and M) may therefore be regarded as opponents to the contractile mass, their function being to maintain the form of the muscle. For this purpose they may therefore be supposed to possess a certain elasticity. The Q and J discs, on the other hand, are the true contractile substances. In so far as units like them are wanting in smooth muscle, they do not represent indispensable contractile elements. The occasion for the segmental arrangement of cardiac and skeletal muscle has been supposed to be the need for

greater speed of action on their part as against that of smooth muscle. Breaking the fiber up into small compartments accomplishes this end, so it has been thought, on account of the increase in surface which thereupon takes place and the consequent increased opportunity for chemical interchange.

(*d*) SARCOMERE

A sarcomere, or, as Heidenhain calls it, a "Komma" or "Inokomma," is contained between two Z lines. Its middle portion is the Q disc and its ends are composed each of one-half of a J disc. Usually the Q disc equals the J disc in height. In the resting position the J disc is equal to about twice the height of Q. Whether these dimensions can be regarded as correct is open to question. Jordan (1920) would perhaps regard a relation between the two discs such as this as being a description of their state in the stretched rather than the relaxed or resting position. The two discs present numerous differences. Both in stained and in unstained preparations, that one is dark (Q) and one light (J) is quite obvious. It is also clear that J appears to be homogeneous, while Q is a more differentiated structure. Chemically they are correspondingly unlike.

There is a difference of opinion as to the water concentration in the two discs. In Heidenhain's view, as likewise in Engelmann's (1878) and Schafer's (1912), J contains the larger amount of fluid. In Jordan's (1920) it is Q. Heidenhain sets up the following differences between Q and J:

1. On drying J appears, wherefore it must contain more water.
2. J refracts light less than Q.
3. J is homogeneous and refracts light more like sarcoplasm.
4. J stretches more easily than Q.
5. With alcohol J shrinks the more.
6. The resistance of J is less, wherefore fragmentation takes place here.
7. J swells less than Q, Q containing more of water-imbibing substances.
8. J stains less deeply.
9. J is isotropic, while Q is anisotropic. The content of anisotropic substance is, however, relative rather than absolute.

The height of a "Komma" or sarcomere in the human heart and in most vertebrates is 2μ. There is in all probability no relation between the size of the "Komma" and the rapidity of contraction, in the sense that the smaller the "Komma" the more rapid the motion, for their height in the wing muscle in flies is great, while that of the muscle in tortoises is very small.

Von Ebner (1920) gives the measurements of the sarcomeres as having a minimum of 2.2μ to 3.0μ, a maximum of 4μ to 5μ, the average being 2.5μ. Q alone equals 1.1μ to 1.25μ. In his opinion Q is homogeneous and contains no H line, at least in the resting state. In this respect Jordan (1920) and

others agree with him. In the resting extended state the measurement may be between 3μ and 5μ (v. Ebner, 1920). Marcus (1921) gives these measurements: When contracted the sarcomere measures 1.3μ; when at rest, 2.2μ. When contracted Q and J have the same height.

Hürthle and Wachholder (1922) found the measurement of the Q and J discs together, of the living frog's auricle in diastole to be 2.2μ to 2.8μ, although in occasional preparations it may attain 4μ, probably as the result of stretching. Q on the average is a little taller than J. They saw the alternation between Q and J discs regularly during diastole. Single and double refracting substance corresponded respectively to J and Q discs.

The description of cross striation so far given is an interpretation which depends on the fact that this appearance results from differentiation within the substance of the fibrils and without reference to the histology of the sheath (sarcolemma); or if this view is not explicitly taken, a distinction between sheath and fibril from the point of view of cross striation is ignored.

Another view is, however, possible and is held by v. Ebner (1920) and by Marcus (1925). According to v. Ebner the appearance of striation depends upon the arrangement of granules; that is to say, of sarcosomes which lie outside the fibril. The fibrils are believed to be longitudinally homogeneous, structureless. Sarcosomes are found in the sarcoplasm but most intimately applied to the surface of the fibrils. The surface layer of fibrils v. Ebner regards as a condensed portion of sarcoplasm, which he calls the pseudo-sarcolemma, being of the opinion that a true sarcolemma does not exist. It is close to this structure that the sarcosomes lie. It is on account of their close application to the fibrils and on account of their presence that the distinction between what is contributed to the appearance of cross striation by them and by the fibrils becomes difficult. If there is striation of the myofibrils at all, this in v. Ebner's view can take place only in the resting state and only because the sarcosomes and the sarcoplastic sheath are responsible for the alternating arrangement through changing processes in these structures both at rest and in the stage of contraction. Von Ebner goes so far as to say that he has never obtained a pure fibril stain, for no matter how easily the fibrils stain the sarcosomes stain more easily still, and more intensively. In progressive staining with hematoxylin the substances which are affected are nodes and streaks which can be followed to the free surface of the fiber where only the sarcosomes and the pseudo-sarcolemma are found. A similar position is taken by Marcus.

(e) N AND E DISCS

The N and E discs are relatively speaking unimportant and on the whole inconstant in human heart muscle. The N disc is made of granules of a substance like that which composes Q discs. Its genetics are unknown and it is

not always present, even in the leg muscle of *Hydrophilus*. It is located, when present, near the z line from which it is separated by the E disc. Finally there is the E disc, a light band described by Engelmann, which separates the z line from the N disc. At about the level of the N disc is to be found the Golgi-Holmgren network, or canalicular apparatus.

3. *Sarcosomes:*

Sarcosomes are of two varieties, the J granules and the Q granules. These are called true granules to distinguish them from fat granules (Bullard, 1912). They have elicited the special interest of Holmgren (1910) and in more recent times have been studied by v. Ebner (1920). The J *granules* were thought by Engelmann and Rollet to be responsible for the appearance of the end disc E. Retzius (1890) found, however, that the granules were not firmly attached to the heart muscle and could be shown to fall away. He therefore denied the existence of the end discs. Holmgren (1910) examined only herbivorous animals, crustaceans and certain insects. He divided granules into endoplasmic and exoplasmic ones, the latter being true granules (Bullard). Exoplasmic granules correspond to Q granules. For convenience and accuracy in describing these structures Holmgren divides the contractile process into the stages of contraction, regeneration, post-regeneration and the facultative stage. In the first stage the sarcomeres are low, the fibrils thick and cross striations not specifically stained except for the contraction band. The granules are seen to be interfibrillary. They separate off from the contraction band and divide between the fibrils. In the regeneration stage the sarcomeres are of the same height as in the first stage. The striations are not specifically stained; the contraction band is paler but distinct. The Q granules show a winged form and are found at the level of the Q disc. Being a phase of active contraction, changes take place more rapidly so that the forms are unstable and are difficult to recognize. In the post-regeneration stage the sarcomeres are of the same height as in the fourth one. Now the granules are easily seen opposite the Q discs. They are round or oval, seem sometimes to be divided or at least to give the appearance of being divided, for at their mid-level there may be a pale zone. In the facultative stage the Q discs are a little thicker than the J. z is pale. The Q granules are found in their typical position. They may be homogeneous, vacuolated or contain pale vacuoles. The Q and J granules, according to Holmgren, are specific organs. Since he believes that substances find their way into fibrils at the level of the z line, he sees a relation to nutrition on the part of the granules in the fact that they resemble the z line in their staining reactions. Holmgren has accordingly proposed that the z line be called "plasmophore."

It is now thought by Heidenhain, who agrees with Holmgren and Retzius, that the contraction band, presently to be described, results in

part from the transfer of J granules to the neighborhood of the Z line. Heidenhain is unwilling to commit himself as to whether there is an end disc apart from the J granules. Jordan (1920) states that the granules take their origin from the Z line and wander between the sarcostyles, that is to say, the myofibrils, towards the level of M. In their course they become gradually rounded as the result of pressure. They are continuously removed and re-formed, their function being one of nutrition. The attribution of this function to them is apparently associated with Jordan's idea that fluid substances enter myofibrils along the level of the Z line. Jordan differs from Retzius, Holmgren and Heidenhain in their opinion that these granules contribute to the formation of a contraction band, for these, as he says, are soluble in alcohol whereas the contraction band is not. He differs from them also in another respect, in that the granules are inter-sarcostylic, whereas the contraction band is exclusively contained within the myofibril. Sarcosomes are apparently of several varieties but those of the wing muscle in wasps (Jordan, 1920) may be preserved in alcohol. These likewise may persist throughout life and always take on a dark stain. Jordan thinks that all muscles contain homologues of the sarcosome found in the muscles of insects. From mitochondria they differ both in shape and in their solubility. Elsewhere Jordan (1919) says that sarcosomes are "more closely analogous to fat globules than to mitochondria." The genetic relation of mitochondria sarcosomes and fat globules remains undetermined. In v. Ebner's drawing, especially in transverse sections, sarcosomes appear to lie between the fibrils. According to him they are found in strings, the strings being so arranged as to include within them single or several myofibrils. Occasionally there are granules in otherwise free sarcoplasm. They measure 0.2μ to 0.3μ. Von Ebner (1920) thinks they do not appear in heart muscle.

The significance of Q *granules* was apparently discovered by Holmgren (1907). It is not yet clear, according to Heidenhain, whether these are the same as J granules. According to Jordan (1920) they are oval, whereas J granules are round. They have their origin presumably in the Z line and are first small granules and later in their passage towards the Q disc increase in size. Their exact morphology and topography and function are not known. Nutritive substances such as glycogen are supposed by some observers (Arnold, 1899) to be transported to the tissue through their medium. According to v. Ebner the granules are small when they are found in the Q disc, where they have an angular or rather spherical form.

4. *Intercalated discs:*

The intercalated discs are one of the two distinguishing characteristics of heart muscle, its syncytial arrangement being the other. A single case seems to have been found in voluntary striped muscle by Jordan (1919).

He cites and discusses a further reference by Dietrich (1910). They have been the occasion of much discussion, both from the point of view of their exact histology as well as from that of their significance. Briefly they are darkly staining bands which occur at irregular intervals in the course of the muscle fiber and measure from 1μ to 1.7μ (Heidenhain). They are not to be found in fetal hearts or in the hearts of children (Cohn, 1909). In the usual methods of staining it can often be demonstrated that they are composed of parallel rods separated by a substance much more lightly stained and that they are continuous with the muscle fibrils. Of the theories both of their structure and function which have been proposed the following may be mentioned: First there is the simple theory that they represent true cell boundaries (Schweigger-Seidel, 1870; Eberth, 1866; Zimmermann, 1910; v. Palczewska, 1910; Werner, 1910). This view of them is, in general, no longer held. There is but one point to be raised in its favor (Jordan and Banks, 1917); namely, that it is at their level that heart muscle fractures (segmentation). The modifications of this theory by Hoche (1897) and Marceau (1904) deserve mention. Hoche regards intercalated discs as being bands of rods already existent before the time of death, that is to say, structures not developed during the process of death or during that of histological fixation. In this view they are not complete barriers for he thinks that the rods were continuous with myofibrils and that sarcoplasm is able to pass through the discs from one to the next neighboring sarcomere. In so far as one sometimes sees a contraction wave end at their level he thinks they have a function in separating active from non-active tissue. Marceau notices their absence in the new-born and states that they develop with advancing years. Their function is to equalize uneven contraction in the heart in the sense that active and non-active portions may be seen to be separated by them, as Hoche pointed out. It is interesting to notice that on an anatomical basis very like this a current notion of the mechanism of the pulsus alternans is based. Marceau regards intercalated discs as developing from J discs. Marceau as well as Renaut and Mollard (1905) think that they are of a tendinous nature. Associated with Marceau's theory is that of Dietrich (1910) who regards them as developing late in the process of histogenesis. It should be pointed out, however, that they do not stain like tendinous tissue.

It is Heidenhain's idea, as has already been pointed out, that they are growth centers. He came on this notion as the result of the necessity which he saw of discovering a mechanism for longitudinal growth and because he found no other way in which the end could be accomplished. A certain support is lent to his theory by the fact that the intercalated discs are often found in regions where fibers divide laterally both in youth and during the pathological process of hypertrophy. With their general arrangement

described by Heidenhain, Dietrich agrees except that he does not subscribe to their function as growth centers.

An important theory is that of v. Ebner (1900 1903) and of Aschoff (1906), which states that intercalated discs result from physical influences which develop perhaps in the course of life, but more likely in the agonal period during the course of contraction. The original idea was that they are influenced by such irregular processes as cramps and fibrillation, which suggests that the agonal process is responsible for their development, especially in old age. In this form the theory is perhaps untenable, but in the sense that these structures represent the result of stresses and strains at points of division of trabeculae and that these increase or at least show the effect of this sort of continuous action during the course of life, the idea has force. The distribution of the intercalated discs speaks for this, that is to say, their increasing frequency beginning with the auricles, then the right ventricle, the left ventricle and finally the papillary muscles. A difficulty in this view concerns the explanation of how structures increase in number and become more complex in the course of life. But in general that this should take place is in accordance with accepted belief; change in the form of many other cells may be cited as the result of comparable processes. This is, indeed, the method of differentiation. Here may be mentioned in the heart itself the gradual accumulation of pigment, resulting ultimately in the appearance called brown atrophy. Closely associated with this theory of development is that which Jordan and Banks (1917) propose.

Certain gross facts in respect to the intercalated discs should be pointed out. They do not in all probability exist in the human fetal heart. Witte (1919) saw them in the heart muscle of fetal pigs as early as the 80 mm. stage. Jordan and Banks (1917) saw them in the beef heart at the second to the third month. Jordan and Steele (1912) saw them in the fourth month and state that they were abundant at seven months. They likewise saw them in guinea pigs during the last week of gestation. In the beef heart, Jordan and Banks state that at two months the cells are plain and fusiform and contain oval vesicular nuclei centrally placed. Some of these divide amitotically. The z lines are present but the q discs are faint and are to be seen only in the fibrils peripherally placed. The fusiform cells begin to coalesce and to form the later network. The intercalated discs arise at the lines of fusion and take a position at right angles to them. At these places a re-alignment of the myofibrils takes place and "the stresses involved may effect the multiplication of the myofibrils which constitute the intercalated discs." Marceau (1904) failed to see them in the new-born. They were seen in one case twenty-four hours old (Cohn, 1909) and in 12 other cases among the new-born. The fact is insisted upon by Aschoff and Tawara (1906) and by Cohn that they occur with increasing frequency with time but that they are most numerous in old age. A similar statement is made by Jordan and

Banks. A study of a large amount of material does in point of fact give the impression that the age of a subject may be told, of course with a latitude of considerable error, from the number of the discs which are present in the heart muscle. It was pointed out, furthermore, that there are fewer in the auricles than in the ventricles. In the auricles Cohn saw an indefinite one only once in a child less than one year old. He points out that they are more frequent in the left than in the right ventricle and most frequent in the papillary muscles. These differences in number between the two ventricles on the one hand and between the auricles and the ventricles on the other Jordan and Banks (1917) failed to find. Their observations apply, however, to beef rather than to human hearts. That in the new-born, on account of its greater functional activity, they might be more frequent in the right than in the left ventricle was not substantiated on a search through numerous specimens (Cohn). In the auricles they appear to be less high than in the ventricles and indeed often have a thickness no greater than that of z lines. The variety of form which is seen in the ventricles is likewise seen in the auricles.

In order to establish the fact that intercalated discs are present in the heart as a part of its normal development, Dietrich (1910) proposes that a theory adopting this position obey four requirements: First, the discs must be found in every heart irrespective of its state of contraction or the duration of the agonal period through which it has passed. Second, they must appear in a characteristic arrangement. Third, there must be a relation between the embryology of the heart and its growth even under pathological conditions. Fourth, there should be set up a natural unforced plan for the significance of their function. To all of these requirements except the fourth Dietrich was able to give satisfactory answers. The answer to the fourth requirement is naturally the least satisfactory, for in an organ so complex as the heart a correlation between function and specific form is still necessarily difficult. Dietrich's idea that the intercalated discs have an important bearing on the derivation of secondary from major trabeculae is important and finds support in Aschoff's general position and in that of Jordan and Banks.

A consideration of these theories, then, shows that certain ground has been gained in the course of the investigations of the last twenty-five years. As the result of histological study it has been possible to relinquish the cell boundary theory, the theory of the tendinous nature of the discs, their significance for growth and the theory of their exclusive origin at the moment of death. The elimination of these ideas provides for more concentrated consideration of the data which are left. These include the absence of the discs during fetal life, their sparse presence in the first years and their increase in number in the course of life, the relation of their distribution so far as number is concerned to those locations in the heart which are exposed

to the highest blood pressure and their existence predominantly at those points within the tissue at which the trabeculae divide and are subject in all probability to the greatest stress. A theory of the intercalated discs may, then, state that they are structures which occur with time and are dependent for their development on the mechanical stresses to which the organ is subject. We come, then, to a consideration first of their histology in detail and second to an interpretation of their histogenesis.

Intercalated discs always appear to be associated with the z band. Their occurrence is not an artefact for they appear both in fresh and in stained sections. Being more or less irregularly spaced and passing as they do on occasion across more than one fiber, a pair of discs often includes irregular fiber territories. Within these territories there is usually one, but sometimes more than one nucleus. Within a territory which embraces two adjacent fibers it sometimes happens that the nuclei are in the one and not in the other fiber. In this case the part of the territory which occurs in the second fiber may be regarded as a wing of the first. It was Heidenhain's idea that the discs are bounded on each side by z lines and occupy the space of an entire sarcomere. According to Jordan and Banks (1917) and to v. Ebner (1920), on the other hand, an intercalated disc is originally bisected by a z line, as is shown by the fact that the sarcolemma when it is observed in the festooned state is indented at the point of a z line (Fig. 356). A disc, on the other hand, may shade into a z line (Fig. 357) or z may be found at the mid-level of an intercalated disc, a proof of the fact that this bisects the disc (Fig. 354).

In the view of Jordan and Banks, intercalated discs are peripheral structures in that on transverse section they do not pass plate-like through the substance of the fibrils but are found either on their surface or at various depths within, as they would be if they were spiral lamellae. In this case, however, the spirals appear to be discontinuous, broken bands rather than continuous structures (Fig. 358). The fact that the discs are superficial, is indicated in Figure 359 where it appears that one passes across the upper pole of a nucleus. An intercalated disc may in longitudinal sections pass entirely across a fiber or across only a fraction. It has already been pointed out that z lines often pass across several fibers; so in the same way may an intercalated disc pass. Discs may be, however, and often are, more complicated. In their passage across a fiber they may break into the form of steps. The step may pass in one direction only or, having proceeded in one, may change its direction and may indeed do so more than once in the course of its passage across the fiber (Fig. 356). For the most part in such arrangements the right border of one segment is in the same vertical line with the left border of the next succeeding one. Occasionally, indeed frequently, intercalated discs are found, as has already been mentioned in discussing the theory of their occurrence, where trabeculae divide (Fig. 356).

In step-like discs it is frequently found, as was pointed out by Cohn (1909), by Jordan and Banks (1917) and by others, that there is a dislocation of the z line in certain cases in the way which is illustrated in Figure 360. The z lines 1 to 8 retain their initial position, but z lines 2′ to 8′ are dislocated. There are other curious appearances which give rise to the idea that in the course of their formation the fibers have undergone twisting (Fig. 361). An effect of this on the sarcolemma, on a shifting of z lines so that they come to look like a Vernier scale (Heidenhain, 1911, 1918) is seen in Figure 362.

In all probability intercalated discs begin as the simplest of the forms described and take on the more complicated appearances such as the steps, the spiral and twisted forms with increase in years, with developmental anomalies (Heidenhain, 1911) and perhaps as the result of mechanical needs as in hypertrophy (Jordan, 1912). On the other hand, in atrophied hearts, as Jordan (1912) has shown, the predominant form of the discs is a simple straight line having what is called the comb form.

In the muscle of the auricles Jordan and Banks (1917) show additional intercalated discs of forms which add varieties to those already described. First is a form of disc in which two adjacent z lines have attached to them, within what is obviously the same sarcomere, two intercalated discs each apparently only partially formed and each distinct from the other (Fig. 363). Then there is a form of disc which ends in two z lines (Fig. 364) and which gives, according to Jordan and Banks, the appearance of that form of differentiation which Heidenhain would have attributed to growth. There is, however, another method of interpreting this figure; namely, that it represents the left half of the fiber as being in a state of contraction, while the right is in relaxation.

Von Ebner (1920) says that in completely dilated resting hearts the intercalated discs which he calls "Glanzstreifen" are usually high, strongly refracting elements which are composed of z lines and the adjacent halves of j. In this he agrees with Jordan and Banks. It has the same refractive index as z but a higher one than q. It will be noticed that v. Ebner differs from Heidenhain in respect to the constitution of the disc, its arrangement according to the latter extending between two z lines. Von Ebner agrees that it is formed of parallel rods. He indicates arrangements in accordance with which intercalated discs may present wedge forms, the point of a wedge being continuous with a z line or more particularly a c band. (The meaning and structure of the c band is discussed under the heading of the histology of contraction.) There are, however, other forms which v. Ebner draws and which are more difficult to understand. In one case an intercalated disc occupies, at least for a fraction of the diameter of a fibril, a height equal to the z line, or more appropriately in this case the c line (to be explained later) and in its second fraction extends through the adjacent half

of J and the whole of the Q disc associated with it (Fig. 365). As has just been indicated, v. Ebner describes intercalated discs as fading into C bands, that is to say, contraction bands. So far as histogenesis is concerned others, especially Jordan and Banks, also identify intercalated discs with these bands. These must now, therefore, be described.

5. Contraction and the contraction band:

It has long been known (Engelmann, 18–8) that during the contractile act there takes place what has been called a reversal of striation. Whereas ordinarily Q discs are dark and J discs light, during contraction that portion of the muscle fibril usually called Q becomes light and a portion, at least in the region of Z, dark. Apparently the first act during the process, certainly in so far as it is identified in the striated muscle in insects, takes place at the mid-level of the Q disc (Fig. 365). This region becomes progressively lighter and in the stage of semi-contraction the ends of the Q disc appreciably darker. When the muscle is completely contracted the H disc which has come to extend over more and more of the region originally occupied by Q appears to be, relatively speaking, tall. Across what was initially the center of Q there now appears the M line generally described as being granular. Simultaneous with the change at the level of Q, change also takes place at the level of Z. At and about Z there assemble the darkly staining ends of Q or more accurately both the nearer-lying halves of two adjacent Q discs as well as the J portion of the corresponding sarcomeres. Included within the new contraction band, C or CB, are also N and E discs likewise collected from the two adjacent sarcomeres.

The constitution of the contraction band may be recapitulated. It is composed of half the Q disc of one sarcomere, J, N and E discs of the same sarcomere, a Z line, E, N and J discs of the next sarcomere and half the Q disc of this sarcomere. A contraction band, according to Jordan (1920), and a simple intercalated disc are identical. An intercalated disc need be composed, however, of no more than half a contraction band beginning, that is, with half of one Q only and ending with Z. In this case it is bounded only on one side by Z. If it is made of two such elements in such a way that the second one is the other half of the same sarcomere which contains the first Q, then the intercalated disc is bounded on both sides by Z lines.

The difference between a contraction band and an intercalated disc, according to the theory of Jordan and Banks, is this: an intercalated disc represents the irreversible state of the contraction band. To describe how such structures arise, why they are irreversible states, has so far not been successfully attempted. Their location so frequently at points of division of trabeculae has already been mentioned. This circumstance as well as others indicates that there are mechanical factors which have to do with their occurrence. That these factors are precisely the ones which might be

expected to develop with age and its increasing demands are matters which have already been described in discussing the theory of these bands.

It remains only to report certain additional observations on these structures. It has frequently been observed that in a contraction wave the height of cross-striated discs decreases continuously until an intercalated disc is reached. The transition to discs of resting height on the other side of an intercalated disc is sudden. This curious appearance has given rise to the idea that intercalated discs offer an inhibition to the spread of contraction waves, but it frequently happens also that one is found in the middle of such an area as well as in areas in which the muscle is at rest. No adequate explanation has yet been offered for this appearance. Hürthle (1909) has seen it in the wings of *Hydrophilus*, although here he regarded it as an abnormal contraction. This appearance was the occasion for Imschanitzky's (1906) idea that the conduction of impulses in heart muscle must be neurogenic in origin. A curious observation was made by Cohn (1909) to the effect that heart muscle which has been stained with Soudan III and in which numerous intercalated discs were present failed to reveal fat droplets in the discs. Similar observations have been made by P. Hoffmann (1909).

6. Certain physical and chemical changes in contraction (*also* Sect. XXII):

During the course of contraction anisotropic substance does not, in Jordan's (1920) opinion, change its location from the Q to the J disc or more especially to the contraction band. As will be pointed out in the discussion on anisotropy, this substance does not contribute to the staining reaction of muscle tissue. It is therefore from this point of view not concerned in the process of reversal of striation. On the other hand, the degree of anisotropy lessens during the contractile act. It was Ranvier who pointed out that other substances such as rubber and glass beside muscle change the degree of their anisotropy in states of greater tension. They are isotropic when unstretched and anisotropic when stretched. Isotropy and the reversal of striation are accordingly not associated phenomena.

It has usually been assumed that in the course of contraction there is a shift of water from the J to the Q disc on the supposition that the light disc J is, relatively speaking, more fluid than the dark disc Q, that is to say, from the direction of Z to M. This is the view both of Engelmann (1878) and Schafer (1912). In the view of Hürthle (1909), however, the state of affairs is reversed (Fig. 366). Jordan (1920) thinks Q is the more fluid portion of the fiber and like Hürthle that fluid passes, therefore, from Q to the J disc, that is to say, in the direction from M to Z. Jordan attempts to substantiate his view by a reference to the order in which the Q disc clears during the course of the formation of the H disc. If fluid passed from the direction of Z to M it ought to be the ends of Q which first become pale as

the result of dilution but instead, as he says, it is the center of Q which does so and the ends of the disc which become darker. How this problem can be solved seems still to be an open question, for it is perhaps necessary to locate with a greater degree of accuracy precisely where in the course of the fiber the concentration of fluid is greater before a final judgment as to the shift of fluid can be accepted. The evidence so far accumulated (Jordan, 1920) indicates that on dehydration it is the Q disc that shrinks, but at the same time it is stated that in hypotonic solution it swells. Experiments such as this seem to give evidence on the concentration of salts rather than on the concentration of water.

The effect of mechanical tension tends to result in stretching the Q disc rather than the rest of the fiber. Jordan (1920) has drawn a diagram to show that stretching does not result in splitting the Q disc and in bringing about the formation of H but rather in the elongation of Q. It is only when the muscle is actually contracted and the H disc is already present that stretching results in elongating this disc. There is likewise, according to Menten (1909), quoted by Jordan (1920), movement of chlorides, phosphates and potassium salts away from the region of M.

In a general way v. Ebner (1920) is of the same opinion as to the constitution of the contraction band as Jordan (1920). On account of its relatively small height, however, 0.7μ, he wonders that it can contain, beside Z, two adjacent J discs, for in the resting state two J discs plus the Z line measure 1.25μ. In accordance with v. Ebner's general views respecting transverse striation is his observation that the substance in contraction bands which stains as well as that in the M line which arises anew is due to sarcosomes. This opinion is shared by Marcus (1925).

This question of the location of water and of its shifts takes on increasing difficulty on account of the limitation of the technique which is available, for whether the tissue is frozen or put into salt solution of various concentrations or into alcohol, new distribution of the water necessarily takes place. It is on account of reasons such as these that the views of Engelmann and Hürthle are divergent. Engelmann's evidence was, of course, histological. In the process of drying or during fixation intrafibrillary water may pass from J to Q and from the interfibrillary sarcoplasm to intrafibrillary sarcoplasm. The fibrils then swell and shorten. That lactic acid plays a part in bringing about a movement of water is maintained by Meigs (1910). In dying muscle, on the other hand, water may pass out from the fibrils and be found under the sarcolemma. Apparently substances which are double-refracting are poorer in water and have the ability to swell to a greater extent than single-refracting ones. Even dead muscle is said in its anisotropic portions to be able to take up water and to shorten like living muscle. The change in the degree of anisotropy in the fibrils during contraction demonstrates the fact that swelling takes place within the anisotropic rods

and since, as Hürthle has shown, there is no volume change, the alteration must be due to redistribution of water within the ultra-microscopic parts of the rods.

The method by means of which contraction takes place has been studied by Hürthle (1909) in measurements of the muscle of *Hydrophilus*. Hürthle shows that shortening takes place only in the Q disc (Fig. 366). His calculations, moreover, indicate that although shortening takes place no change in volume occurs. The J disc, instead of shortening, actually lengthens. Obviously the transverse measurement of the fiber increases. Since in these measurements the Q disc and the sarcoplasm together decrease about 15 per cent, the decrease must take place in the sarcoplasm, for the rods in Q remain unchanged. Since the J disc increases in height Hürthle presumes that sarcoplasm must pass from Q to J, contrary, as has been said, to Engelmann, but in accordance with Jordan. Objections have been raised to this interpretation by Meigs (1910, 1914) by Schafer (1910) and McDougall (1910); in v. Fürth's (1919) opinion these measurements of Hürthle nevertheless deserve credence.

7. *Sarcolemma:*

On the existence and the nature of the sarcolemma there is considerable disagreement. Viewed uncritically the sarcolemma is a sheath which surrounds the fibrils and is derived from the perimysium, a connective tissue structure. On this supposition it is, of course, independent genetically of the muscle fibrils. This, on the whole, is Heidenhain's and the generally accepted view. There is another view, however, supported by v. Ebner (1920), Marcus (1925) and Schafer (1912) according to which the independent existence of the sarcolemma is an illusion; it depends really on an appearance taken on by the most superficial layers of the fibrils. There is a further question as to whether the sarcolemma is a homogeneous membrane or whether it is finely reticulated. Those who describe a true sarcolemma agree that there is an intimate connection between this sheath and the z line as well as with contraction bands and intercalated discs. Frequently in the case of contracted muscle, when there is shortening of the Q discs, the attachment of the sarcolemma to the z line indents the membrane so that it appears to be festooned. But at the level of the intercalated discs it lies flat even when elsewhere it is festooned. Usually the z lines of neighboring fibers lie at the same level. But there are numerous instances in which dislocation of half a disc or less takes place. Especially in these cases, as Jordan and others have shown, it is instructive to follow the connections of z lines, for they can be seen to pass from one fibril across to another in an oblique direction (Fig. 361). In these observations, evidence is gained, first, that there is a sarcolemma and, second, that z connections pass across interfibrillary (sarcoplasmic) spaces.

When this arrangement of the sarcolemma takes place, there is found an unequal number of sarcomeres in the two columns, so joined. The appearance which results is described by Heidenhain as a Nonius period, or, we should say, as a Vernier scale. Its origin is differently given. Heidenhain (1911) suggests that it might result from twisting of the fibrils and gives diagrams (Fig. 353, p. 616) to illustrate this thought. More recently Heidenhain (1912) has devoted attention afresh to the meaning of the so-called Nonius fields. Where they occur, at least in the sternohyoid muscle in dogs, they form part of a larger arrangement. A Nonius field appears as a wedge-shaped body inserted into the mass. Obviously the insertion of a wedge such as this disturbs the general arrangement of the sarcomeres so that one border of the whole mass, the one toward which the apex is turned, becomes much shorter than the other border unless a correction is made, the whole fiber being bent. The correction is made by the insertion of another wedge, the apex of which is pointed in the opposite direction. If the fiber mass contains ten fibers on longitudinal section, a wedge such as is described is constituted so that fiber one consists of one sarcomere; fiber two, of two; fiber three, of three, etc. Obviously the sarcomeres of adjacent fibers are on slightly different levels; each pair forms a Nonius field in a restricted sense. The entire fiber mass of ten, each fiber containing an additional sarcomere, forms the field. This is but one form in which the rearrangement of what Heidenhain calls the "Zwischensarkolemma" takes place. To correspond to this description there need not necessarily be dislocation of the sarcomeres, such as has been described. Stretches of no more than two or three sarcomeres may be involved. How they come into being in heart muscle sections is not so difficult to conceive. They may arise as the result of mechanical relations either of twisting or of some other stress between the adjacent fibers. Heidenhain also suggests the possibility that a developmental anomaly may be the cause.

Iwanaga (1925) describes the development of the sarcolemma in the striated muscle of the extremities in human beings. Before him it had been studied in human tissue by Pappenheimer (1908) and Schiefferdecker (1909). According to Asai (1914), the sarcolemma in rabbits arises from myoblastic protoplasm and originally forms a thin homogeneous membrane. Later a reticulated connective tissue membrane surrounds it when according to his view, nuclei begin to wander out from the axis of the fibers and take up their position under the surface then the definitive sarcolemma is developed. It is visible but still little differentiated from sarcoplasm. Iwanaga's studies in human beings agree with those of Asai. He has found the first sign in embryos 5.8 cm. long, where it can be recognized only on transverse section at the time when the myofibrils are arranged circumferentially either in a ring or half ring. The ring is surrounded by a most delicate membrane formed of protoplasm. This then becomes thicker so

that by the time the embryo has attained the length of 15.5 to 19 cm. it can safely be regarded as a sarcolemma. At the age of eight months it can easily be detected.

Marcus's (1925) view is that the sarcolemma is not homogeneous but is a network which is not separable from the perimysium, and that it is fastened to the cardiac fiber by extensions which penetrate this structure in the elaborate way already described. Von Ebner states that the sarcolemma is an artefact brought about by the action of agents on the surface of the fibers, a mere adsorption phenomenon. He says that he has seen similar appearances at the edges of fractured muscle. Sometimes at the level of the z lines the sarcosomes which often have wing-like projections occasion an appearance which is mistaken for a sarcolemma and which accordingly he calls a pseudo-sarcolemma.

8. *Sarcoplasm:*

Sarcoplasm is a structureless substance, perhaps more fluid in nature than the fibrils themselves, and lies between them. It is the substance which separates the fibrils from each other and in which the granules lie. Sarcoplasm is apparently difficult to demonstrate for in well-fixed specimens a minimal amount of space exists between the fibrils. That there is such a substance is asserted especially on the basis of the fact that silver impregnations bring out a network surrounding groups of fibrils (Cohnheim's fields, 1865), which is seen on transverse sections. Whether there is also an intrafibrillary substance of similar nature is not known. At all events Hürthle's (1907) experiment, in which he centrifuged muscle fibers with the view by this force of emptying or of dislocating the contents of the fibrils, failed. If fluid substances are contained within the fibrils it may perhaps be that they are retained in place by such structures as the z lines.

9. *Refracting substance:*

No detailed description before now has been attempted of the subject of the refraction of muscle substances. The relation of this property to the striated appearance of muscle has usually been regarded as intimate. The Q discs were described as anisotropic and the J disc as isotropic. In view of the fact, however, that there is accumulating evidence to show that double refracting anistotropic substance is distributed throughout the sarcomere and that there appear to be only relative differences in concentration at different levels, it is perhaps better to dissociate its description from the description of cross striation. This separation seems on the whole the more to be commended in view of the fact that the substances which stain are probably not connected with those that show anisotropy. If there were a relation of this nature one would expect that anisotropic substance would in contracting muscle depart from the level of Q discs and become deposited

in the contraction band. This transfer seems, however, not to take place (Jordan, 1920). Those levels at which there are greater concentrations of anisotropic substance seem to be the Q disc and the z line and more especially the z line when it becomes part of the contraction band.

There has been much experimenting on the subject of the nature of double refraction beginning with the experiments and observations of Brücke (1858). These have recently been reviewed by Stübel (1923). Stübel's conclusion is that the degree of double refraction depends on the relation of the specific refraction of a substance to that of the medium in which it is embedded. It is perhaps not necessary in this place to discuss theories of double refraction in detail, more especially since there has been no satisfactory identification of the fact of double refraction with the substances or structures that give rise to it. Stübel states that there are in muscle, arranged in similar fashion, crystalline rods of albumin which are positively double refracting, and crystalline lipoid particles which are negatively double refracting either on account of their nature or on account of their position. The degree of double refraction which is read is a resultant of these two.

In addition to the general statements on the distribution of anisotropy in the sarcomere, there is v. Ebner's observation (1920) that the M disc, when it appears during contraction, is isotropic, whereas the ends of the Q disc, when Q separates during contraction, are double refracting. Von Ebner is interested in the relation of the action of sarcoplasm to that of the structureless (at least to him) myofibril, his view being that it is alteration in the sarcoplasm (sarcosome) which is responsible for the anisotropic appearance.

So far as the relation to contraction is concerned, it appears (Jordan, 1920) that the Q disc loses its anisotropic quality during this act. In this connection Jordan finds evidence for his view that the Q disc is more fluid than the J, for he presumes that the decrease takes place more easily in the more fluid medium.

Other authors also describe the fact that double refraction decreases during contraction (v. Fürth, 1919), as well as during rigor mortis. In tetany it is of a high grade. Double refraction increases because on the basis of v. Ebner's view, which applies in this case, increased tension is associated with increase in the degree of double refraction.

10. *Summary:*

So far this account of the histology of heart muscle has concerned itself with histogenesis and with histology in the adult state. There remain to be considered certain alterations in form which take place during the course of life: the fracture of muscle frequently observed especially in advanced years; regeneration and hypertrophy, manifestations which result from the

attempt on its part to meet the injury of disease; and finally certain well-established degenerations which occur during the course of life.

V. GROWTH

Those changes in the course of life which affect the intercalated discs have already been described. There are several observations, however, which concern *nuclei* that should be mentioned. It is generally agreed that in the fetus and in the early years of life the nuclei are rod-shaped, perhaps somewhat oval or round, and that their surfaces are quite smooth. With increasing years the nuclei are slightly flattened at their poles. Later still there are to be seen, especially on cross section, small longitudinal grooves which give the appearance of irregularity in outline. On the significance of this appearance there was carried on a discussion between Romberg and Inada and Aschoff (1906). In contraction this change is exaggerated; the nuclei have a more rounded form. When the muscle is relaxed there is a tendency for the nuclei to be more elongated. These appearances apparently exist independently of the injury which is done in the course of fixation and embedding. In the hypertrophic state, however, the same change is observed except that a striking exaggeration of the effect takes place. In all probability, then, with increase in age slight alterations in the form of the nucleus take place. When muscle is hypertrophied the nuclei undergo still greater alteration. They appear much swollen, have irregular outlines both on transverse and longitudinal section and appear to have a marked decrease in chromatin.

The space in which the nucleus is found is in the center of the fiber. At first the space is closely approximated in size and shape to that of the nucleus, but soon it becomes fusiform and with advancing years its dimensions increase not so much at the sides as at the two ends. As early as about the tenth year pigment in the form of small granules is laid down here. The granules are yellow and contain lipoid; Aschoff says they are negative for iron. During the course of time its amount progressively increases until there is enough in the sixth and seventh decades to give the muscle the brown color associated with what is termed brown atrophy by pathological anatomists. The occurrence of pigment of this nature is not to be regarded as an accompaniment of disease but as a manifestation of normal growth.

Accompanying these changes the sarcomeres also appear to undergo a change in that they seem to be less high both as regards the J and Q discs, but especially in respect to J. No measurements indicative of this change are available. The phenomenon may, however, be associated with dessication as an accompaniment of senescence. At the same time cross striation appears to become a more marked and striking characteristic.

A difference between the structure of the fibers of auricles and of ventricles may also be mentioned. A difference, so far as intercalated discs is

concerned, has already been described. But there are further differences. In the first place, striation in the auricles is less marked both in the longitudinal and cross varieties. It appears to be a fact, although no measurements are available, that the height of the discs in the auricles, especially that of the J disc, is greater than that in the ventricles. The nuclei are, relatively speaking, large, whereas the perinuclear pigment is less copiously developed. That in which the auricles predominate over the ventricles is in the amount of connective tissue.

VI. SEGMENTATION

A subject which has long been a matter of controversy is the fracture of cardiac muscle fibers. The point of contention has consisted in the attempt to decide whether fracture takes place at the level of the intercalated discs or elsewhere in the course of the fiber. In the first case the phenomenon is called segmentation, in the second fragmentation. A difficulty has existed because the fracture line is frequently irregular. Intercalated discs are, it will be remembered, either straight or terraced. Should fracture of the fiber take place at their level one would anticipate that the line of fracture would vary with the kind of intercalated disc through which the fracture took place. In an investigation of this subject by Saphir and Karsner (1924) the position is taken that the differences between fragmentation and segmentation are apparent rather than real. They make the statement that uniformly the presence of at least part of an intercalated disc can be found at the line of fracture. Browicz (1893), for instance, believed that the intercalated discs were more conspicuous in the early stages of fracture. He, as well as more recently Jordan (1911) and Jordan and Bardin (1913), associated intercalated discs with the development of fracture.

In a study of this subject it seemed (Cohn, 1909) that the fracture took place most frequently in straight lines when the lesion was obviously associated with intercalated discs, but that otherwise the line of rupture was irregular. Exact attention was not paid to the relation of the fractured ends to the possible remnants of terraced intercalated discs. In view of the fact that v. Ebner raises the question of the possibility of absorption of dyes by torn muscle ends, the question may perhaps still remain open, although for the most part evidence seems to be accumulating to indicate that it is actually through the intercalated discs that fracture takes place. Saphir and Karsner found that this phenomenon may take place at any time after the age of twenty. They found only 2 cases in younger individuals.

A reason for rupture is found by them in the supposition that intercalated discs are weakened in those hearts in which segmentation occurs. The fact that the fracture lines are so frequently irregular is attributable to the fact that it is precisely in hearts which are diseased in which terraced

intercalated discs are found. Lesions in the heart and terraced intercalated discs are associated phenomena. If rupture of the fibrils takes place it is to be expected in the first place in diseased hearts and in the second place in the step-like intercalated discs characteristic of them. Disease in this instance refers more especially to hypertrophy (Jordan, 1912). In normal hearts where discs tend to be straight, straight lines of fracture are more usual. In addition to supposing that intercalated discs represent the point of weakness in heart muscle, Saphir and Karsner also suppose that a force such as causes dilatation occasions the rupture. They have in point of fact done experiments in rabbits, cats and dogs, which show this relation. That an unusual force is necessary to bring segmentation about is supported by a personal communication from Aschoff to these authors to the effect that he was able to demonstrate segmentation in soldiers killed in action only after rigor mortis had set in.

The history of this subject (for early references to which Hektoen's 189- paper may be consulted) as well as its significance, such, for instance as that it represents a distinct disease, that it is a technical artefact, that it is responsible for the manifestations of valvular insufficiency, that it occurs in the agonal period or is a post-mortem change—none of these matters need be developed for the purposes of the present presentation.

VII. HYPERTROPHY AND REGENERATION

That hearts depart from a size which is in general established as normal and that the volume of the muscle under these circumstances either increases or decreases has long been known. The problem at issue has centered more especially about the presence of hypertrophy and how the hypertrophic change affects the muscle fibers. Is there an increase in their total number or do the individual fibers enlarge? Edens (1913) has reviewed the numerous statements which observers have made on this subject. It is, for instance, said that the muscle fibers of hypertrophied hearts are thinner than normal; that the heart itself is increased in size as the result of the growth of pre-existing younger cells or of the development of new cells; that the muscle fibers of hypertrophic hearts have a greater diameter than those of normal ones, but that this result may depend either upon increase in the amount of sarcoplasm or in the number of fibrils. Statements have been made that the amount of sarcoplasm is normal, but others state that it is sometimes increased. Connective tissue is said to be increased by some, unchanged by others. The study of Karsner, Saphir and Todd (1925) makes the effort to reduce this confusion to a degree of order by introducing an exact method of observation and of numerical statement. They have studied normal, atrophic and hypertrophic hearts. They counted the number of fibers and the number of nuclei within certain dimensions and measured the breadth of the fibrils (Table 1).

TABLE 1*

	Average breadth of fibers, arbitrary unit	Average number of nuclei in field	Average number of fibers in 0.00015 cu. mm.	Volume	Total number of fibers	Number of fibers per 100 c.c.	Weight, in gms.
1. Normal heart.....	0.503	11.0	11.549	295	22,715,000,000	7,700,000,000	300
2. Atrophic heart.....	0.438	22.7	10.801	160	11,520,000,000	7,200,000,000	165
3. Hypertrophic heart..	0.907	9.8	7.394	485	23,894,010,000	4,926,600,000	500

*This table is combined from data given in Tables by H. T. Karsner, O. Saphir and T. W. Todd, (1925)

Briefly the results of their study may be summarized as follows: Although the volume of the hypertrophic heart is much greater than that of the normal heart, the number of fibers is the same; no hyperplasia of the fibers has taken place. That this is likely is seen by the fact that the breadth of the fibers is almost twice as great in the hypertrophied heart. These relations are further indicated by the fact that the number of fibers per 100 c.c. is reduced. In the atrophic heart, on the other hand, the number of fibers per 100 c.c. is the same as that in the normal heart because the average diameter of the fibers is the same, but the total number of fibers is reduced because the total volume of the heart is smaller. Fibers must in consequence have disappeared in the atrophied heart, a result which is suggested by Bradley (1922). It is a striking fact that the number of nuclei in the atrophic heart is increased, a point which lends support to Eden's (1913) observation that the purine bodies in atrophic hearts are increased, although there is no evidence either of mitotic or amitotic division in the sections. Support for the idea that the nuclei are increased in size but not in number in hypertrophy is gained from the observation of Collier (1922) that the nucleoplasmic ratio is essentially the same in hypertrophic and atrophic hearts. The idea that the fibers in hypertrophic hearts are actually increased in size results from this study of Karsner, Saphir and Todd.

The question of whether regeneration of heart muscle takes place has been difficult to answer. The literature on the subject is small. The occasion for its occurrence is to be found when the heart enlarges as the result of chronic strain, a subject which has already been dealt with in speaking of hypertrophy. Under these circumstances there has in all probability been no destruction of tissue. In diphtheria, on the other hand, the situation is different. In the course of this disease destruction of muscle tissue actually takes place. Repair may occur in several ways: through hypertrophy of the remaining fibers; through hyperplasia by longitudinal division of the fibers which remain and perhaps also through hypertrophy of these fibers; and through the development of new fibers. All these forms may according to Heller (1913 1914) co-exist. In fresh sections he has found spindle cells

with one or two cross striations near the poles of the nucleus and numerous transitions between cells such as these and normal fibers. Heller quotes Waldeyer (1865) as saying that he saw like processes in the hearts of typhoid fever patients. Heller examined a large series of hearts and found that in the first place the muscle which had been destroyed was carried off by the lymph stream and also by phagocytes. The most striking occurrence is the increase in size of the nuclei. These also increase in number in the new fibers. The fibers divide and to these adhere other myogenous cells (compare Meves). Bundles of large size originate in this manner. Heller has published illuminating drawings which show how fibers in which nuclei are contained may split longitudinally. These divided fibers may show cross striation.

Warthin (1925) also has studied the regeneration of heart muscle fibers in cases of diphtheria. The nuclei in his cases show great variety in size and form in the vicinity of regenerating tissue. They increase in length and present evidence of longitudinal splitting. "The living muscle substance bordering on the injured area also undergoes a longitudinal splitting into muscle bands containing nuclei; these bands grow into the perimysial tubes, building these up, replacing the cell detritus, and connecting with the living muscle on the other side of the defect. Muscle bands without nuclei in a substance but accompanied by myoplastic nuclei also extend into the tubes occupied by the dead muscle substance. These bands lie at the periphery of the tubes, and in some cases appear to form a hollow cylinder enclosing the remains of the dead muscle substance. Transition forms from tubes with a single peripheral syncytial layer to those filled with a number of longitudinal bands occur. The myoplastic nuclei are at first large, oval or spindle shaped, staining less deeply than mature heart muscle nuclei, but later becoming contracted and more deeply staining." Transitions between young and mature nuclei also occur. At first there are no striations but later fine ones appear near the poles of the nuclei. Bulbous swellings with many myoplastic nuclei occur at the ends of muscle defects.

VIII. PATHOLOGY

1. *Fatty degeneration:*

With ordinary stains it has been found that droplets of fat, both fine and coarse, are found in the course of heart muscle fibers except at the level of the intercalated discs. Whether fat arises from the degeneration of albuminous substances within muscle fiber or whether it is transported there from elsewhere in the body is an open and much disputed question. Fatty degeneration is usually regarded as the result of resorption or transformation of lipoid substances with which the cell proteins have nothing to do. Aschoff, for instance, thinks that there is very little lipoid substance already in the cell which can be used in the degenerative process. The possi-

bilities, then, are, first, that the fat found in heart muscle is brought thither from other tissues; second, that local causes may operate in the formation of fat, such, for instance, as defects in the circulation resulting in the absence of sufficient oxygen; third, that there may be intracellular causes, such, for instance, as alteration in the activity of fat-splitting ferments; and, fourth, that circulatory disturbances may be present. That diet has an effect on the amount of fat in heart muscle is clearly evident from the fact that there is relative absence of it in animals fed upon fat-poor diets although in the hearts of animals so treated a certain amount is nevertheless present. It can, of course, be enormously increased through fatty diets, the injection of fat into the peritoneum, and so on (Wegelin, 1913). How fat is taken out from the body fluids by the tissues, how they work it up, and precisely how it is utilized are still open questions. One is still not in a position to distinguish fat which is transported to the heart muscle from fat depots elsewhere in the body, from that which arises autochthonously. In addition to this difficulty is the difficulty in distinguishing pathological from physiological cases. It appears, then, that fat is often present in the heart muscle and that it is often unequally distributed. It occurs both in health and disease. It need not necessarily depend upon diet. It may interfere with the normal behavior of contraction, but whether this is so and to what extent are unknown.

2. *Amyloid degeneration:*

Amyloid degeneration, the waxy degeneration of Zenker, apparently occurs infrequently in heart muscle fibers. It is seen in the connective tissue and in the blood vessels and membranes rather than in the heart muscle itself. Occasionally it is supposed to occur in the sarcoplasm which is found between the fibrils. In the muscle itself apparently it does not occur.

3. *Calcification:*

Calcification of the heart muscle fibers is not infrequently found. It begins by being deposited in the form of droplets like fat and is arranged like fat parallel with the fibers. To what structure calcium is bound is not known, but the suggestion has been made that it is attached to the sarcosomes. At first, cross striations remain recognizable, but later their appearance is destroyed, as is also that of the nucleus. Nothing is left but strands of calcium. Single fibers or bundles of them may be involved. The extent of the process, of course, varies. The blood vessels may also be involved. Calcium usually appears in the form of phosphates, much less frequently in that of carbonates. When the fibers are decalcified they are said sometimes to be larger than normal and to appear hyaline. The heart may be the only organ involved in this process. The origin of calcium formation is unknown. It may be due to primary degeneration (Hedinger, 1907; Pap-

penheimer, 1910) but this seems unlikely, for degeneration of the muscle takes place frequently, whereas calcification is rare. Its infrequency is supposed by Hedinger to be due either to the fact that its presence is overlooked or that it is mistaken for some other lesion. The question remains, however, as to how calcium is formed; whether in degenerated or in still healthy fibers. The fact that it is found in fibers which are distinctly cross-striated is not evidence of the fact that they are still normal, for cross striation is seen in fibers in which for other reasons it is known that they are the seat of degenerative processes. Its source under certain circumstances may be bone in which destruction has taken place in diseases such as osteomalacia, sarcoma of the dura and in other similar atrophying conditions. Even so, however, invasion of the heart muscle is rare. Presumably under these circumstances calcium is conveyed to the heart by way of the blood stream. It has been supposed that if at the same time or even independently there is a defect in the kidneys, so that elimination of calcium does not take place in the normal fashion, a rearrangement of the physical chemistry of the plasma takes place so that calcium goes out of solution. In this way excess of calcium may occur in the absence of disease of the bones. Calcium metastases have, indeed, been seen (M. B. Schmidt, 1913) in the veins of other organs, as, for instance, lungs, kidneys and pancreas. But this process again is rare and chronic nephritis is common. When calcium is bound to heart muscle, therefore, a predisposition to its reception on the part of the tissue may be supposed. Calcification is encountered in heart muscle in cases of infection and of intoxication but also independently of these conditions. What has just been said of the relative infrequency of this condition in relation to the frequency of nephritis applies also in the case of acute infections.

IX. BIBLIOGRAPHY

Asai, T. 1914. Beiträge zur Histologie und Histogenese der quergestreiften Muskulatur der Säugetiere. *Arch. f. mikr. Anat.*, **86** (1. Ab.), 8.

Aschoff, L. 1906. Zur Diskussion des Vortrages von Dietrich. *Verh. d. Deut. Pathol. Ges.*, **10**, 45.

Aschoff, L., and Tawara, S. 1906. *Die heutige Lehre von den pathologisch-anatomischen Grundlagen der Herzschwäche.* Jena.

Bradley, H. C. 1922. Autolysis and atrophy. *Physiol. Reviews*, **2**, 415.

Browicz, 1893. Ueber die Bedeutung der Veränderungen der Kittsubstanz der Muskelzellbalken des Herzmuskels. *Arch. pathol. Anat.*, **134**, 1.

Bullard, H. H. 1912. On the interstitial granules and fat droplets of striated muscle. *Am. J. Anat.*, **14**, 1.

Burrows, M. T. 1912 13. Rhythmische Kontraktionen der isolierten Herzmuskelzelle ausserhalb des Organismus. *Münch. Med. Woch.*, **27**, 1473.

Cohn, A. E. 1909. Zur Frage der Kittlinien der Herzmuskulatur. *Verhandl. d. Deut. Pathol. Ges.*, **13**, 182.

Collier, W. D. 1922. Adaptive changes of heart muscle, *J. Med. Res.*, **43**, 207.

Davis, C. L. 1924. The cardiac jelly of the chick embryo. *Anat. Rec.*, **27**, 201.
Dietrich, A. 1910. *Die Elemente des Herzmuskels.* Jena: Gustav Fischer, 46 pp.
Dietrich, H. 1906. Die Querlinien des Herzmuskels. *Verh. d. deut. pathol. Ges.*, **10**, 40.
Duesberg, J. 1909. Ueber Chondriosomen und ihre Verwendung zu Myofibrillen beim Hühnerembryo. *Verb. d. Anat. Ges.*, Anat. Anz., **34**, 123.
Eberth, C. J. 1866. Die Elemente der quergestreiften Muskeln. *Arch. f. pathol. Anat. u. Physiol.*, **37**, 100.
v. Ebner, V. 1900. Über die Kittlinien der Herzmuskelfasern. *Sitzungsber. der Kaiserl. Akad. d. Wiss., Mathem.-naturwiss. Klasse*, cix, III, 700.
——— 1903. Über die natürlichen Enden der Herzmuskelfasern. *Wien. klin. Wochnsschr.*, **4**, 110.
——— 1920. Ueber den feineren Bau der Herzmuskelfasern mit besonderer Rücksicht auf die Glanzstreifen. T. 2, 1 Abt. *Sitz-Ber. Akad. Wiss. Wien. Math. Nat. Kl.*, Abt. 3, **129**, 3 and 93.
Eckhard, C. 1867. Zur Entwickelungsgeschichte der Herzmuskulatur. *Zeit. f. rat. Med.*, 3. Reihe, **29**, 55.
Edens, E. 1913. Ueber Herzhypertrophie. *Deut. Arch. f. klin. Med.*, **111**, 288.
Engelmann, Th. W. 1878. Neue Untersuchungen über die mikroskopischen Vorgänge bei der Muskelcontraktion. *Arch. f. d. ges. Physiol.*, **18**, 1.
v. Fürth, O. 1919. Die Kolloidchemie des Muskels und ihre Beziehungen zur den Problemen der Kontraktion und der Starre. *Ergeb. der Physiol.*, **17**, 362.
Godlewski, E. 1902. Die Entwicklung des Skelett- und Herzmuskelgewebes der Säugetiere. *Arch. f. mikr. Anat.*, **60**, 111.
Hedinger, E. 1907. Ueber Herzverkalkung. *Verb. d. deut. patbol. Ges.*, **11**, 295.
Heidenhain, M. 1899. Beiträge zur Aufklärung des wahren Wesens der faserformigen Differenzierungen. *Anat. Anz.*, **16**, 97.
— — 1911. *Plasma und Zelle.* Jena.
— —— 1918. Die Entdeckung der Noniusfelder in der quergestreiften Muskelfaser. *Anat. Anz.*, **51**, 49.
Hektoen, L. 1898. Segmentation and fragmentation of the myocardium. *Trans. Patbol. Soc. Philadelphia*, **18**, 233. (Also *Amer. Jour. Med. Sci.*, 1897, **114**, 555.)
Heller, A. 1913–14. Ueber Regeneration des Herzmuskels. *Beitr. z. patbol. Anat. u. z. allge. Patbol.*, **57**, 223.
Hoche, A. L. 1897. Recherches sur la structure des fibres musculaires cardiaques. *Bibl. Anat.*, Paris et Nancy, **5**, 159.
Hoffmann, P. 1909. *Ein Beitrag zur Kenntnis der sogenannten Kittlinien der Herzmuskelfasern.* Iwanaga Dissert. Leipzig.
Holmgren, E. 1910. Untersuchungen über die morphologisch nachweisbaren stofflichen Umsetzungen der quergestreiften Muskelfasern. *Arch. f. mikr. Anat.*, **75**, 240.
Hürthle, K. 1907. Ueber die Struktur des quergestreiften Muskels im ruhenden und tätigen Zustande und seinen Aggregatzustand. *Biol. Zentralbl.*, **27**, 112.
—— 1909. Ueber die Struktur der quergestreiften Muskelfasern von Hydrophilus im ruhenden und tätigen Zustand. *Arch. f. d. ges. Physiol.*, **126**, 1.
—— 1914. Erwiederung auf die vorliegende Ansicht von Meigs. *Ibid.*, **158**, 100.
Hürthle, K., and Wachholder, K. 1922. Über die Struktur der Herzmuskelfasern. *Arch. f. d. ges. Physiol.*, **194**, 333.
Imchanitzky, M. 1906. Quelles sont les voies que suit dans le coeur l'excitation motrice? *Arch. internat. d. physiol.*, **4**, 1.
Iwanaga, I. 1925. Beiträge zur Entwickelungsgeschichte der quergestreiften Muskelfasern beim Menschen. *Mitt. allge. Patbol. u. patbol. Anat.*, **2**, 395.

Jordan, H. E. 1911. The structure of the heart muscle of the humming bird with special reference to the intercalated disc. *Anat. Rec.*, **5**, 517.

——— 1912*a*. The intercalated discs of hypertrophied heart muscle. *Ibid.*, **6**, 357.

——— 1912*b*. The intercalated discs of atrophied heart muscle. *Proc. Soc. Exper. Biol. & Med.*, **10**, 42.

——— 1919. Studies on striped muscle structure. IV. *Anat. Rec.*, **16**, 203.

——— 1920. Studies on striped muscle structure. VI. *Am. J. Anat.*, **27**, 1.

Jordan, H. E., and Banks, J. B. 1917. A study of intercalated discs of the heart of the beef. *Am. J. Anat.*, **22**, 285.

Jordan, H. E., and Bardin, J. 1913. The relation of the intercalated discs to the so-called "segmentation" and "fragmentation" of heart muscle. *Anat. Anz.*, **43**, 612.

Jordan, H. E., and Steele, K. B. 1912. A comparative microscopic study of the intercalated discs of vertebrate heart muscle. *Am. J. Anat.*, **13**, 151.

Karsner, H. T., Saphir, O., and Todd, T. W. 1925. The state of the cardiac muscle in hypertrophy and atrophy. *Am. J. Pathol.*, **1**, 351.

Lake, N. C. 1916. Observations upon the growth of tissues in vitro relating to the origin of the heart beat. *J. Physiol.*, **50**, 364.

Lewis, M. R. 1919. Development of cross striations in the heart muscle of the chick embryo. *Johns Hopkins Hospital Bull.*, **30**, 176.

——— 1920. Muscular contraction in tissue culture. *Contributions to Embryology No. 35. Carnegie Inst.*, **9**, 193.

Lewis, W. H. 1923. Cultivation of heart muscle from chick embryos (4 to 11 days) in Locke-bouillon dextrose medium. *Anat. Rec.*, **25**, 111.

MacCallum, J. B. 1897. On the histology and histogenesis of the heart muscle cell. *Anat. Anz.*, **13**, 609.

Marceau, F. 1904. Recherches sur la structure et le développement comparés des fibres cardiaques dans la séries des vertébrés. *Ann. des Sci. Naturelle: Zoologie*, VIIIe Serie **19**, 191.

Marcus, H. 1925. Über den feineren Bau des menschlichen Herzmuskels. I. *Z. wiss. Biol.*, Abt. B., **2**, 203.

Meigs, E. B. 1910. The effects of distilled water of various solutions on the weight and length of striated muscle. *Am. J. Physiol.*, **26**, 192.

——— 1914. Ob die Fibrillen der quergestreiften Muskeln ihr Volum während der Kontraktion verändern? Hürthle's Ergebnisse und ihre Auslegung. *Arch. f. d. ges. Physiol.*, **158**, 92.

Menten, M. L. 1909. The distribution of fat, chlorides, phosphates, potassium and iron in striated muscle. *Trans. Canadian Institute*, **8**, 403. Bound in *Univ. Toronto Studies, Physiol. Series*, 1900–1912, No. 7, 3.

Meves, F. 1907. Ueber Mitochondrien bezw. Chondriokonten in den Zellen junger Embryonen. *Anat. Anz.*, **31**, 399.

——— 1909. Ueber Neubildung quergestreifter Muskelfasern nach Beobachtungen am Hühnerembryo. *Ibid.*, **34**, 161.

Olivo, O. 1925. Sull' inizio della funzione contrattile del cuore e dei miotomi dell' embrione di pollo in rapporto alla loro differenziazione morfologica e strutturale. *Arch. f. exper. Zellforsch.*, **29**, 427.

v. Palczewska, I. 1910. Ueber die Struktur der menschlichen Herzmuskelfasern. *Arch. f. mikr. Anat.*, **75**, 41.

Pappenheimer, A. M. 1910. Calcification of the heart muscle fibers. *Proc. N. Y. Pathol. Soc.*, **10**, 129.

Renaut, J., and Mollard, J. 1905. Le myocarde. *Rev. générale d'Histol.* **1**, 143.

Saphir, O., and Karsner, H. T. 1924. An anatomical and experimental study of segmentation of the myocardium and its relation to the intercalated discs. *J. Med. Res.*, **44**, 539.

Schafer, E. A. 1910. On McDougall's theory of muscular contraction with some remarks on Hürthle's observations on muscle structure and the changes which it undergoes in contraction. *Quart. J. Exper. Physiol.*, **3**, 63.

——— 1912. *Textbook of microscopic anatomy*. New York: Longmans Green and Co.

Schmidt, M. B. 1913. Kalkmetastase und Kalkgicht. *Deut. med. Woch.*, **1**, 59.

Schweigger-Seidel, F. 1870. *Das Herz*. Strickers Handbuch. (S. Stricker. Manual of human and comparative histology. The Heart, by F. Schweiger-Seidel London. **1**, 244.)

Shipley, P. G. 1916. The development of erythrocytes from hemoglobin-free cells and the differentiation of heart fibers in tissue cultivated in plasma. *Anat. Rec.*, **10**, 347.

Stammer, A. 1907. Untersuchungen ueber der Fragmentation und Segmentation des Herzmuskels. *Ziegler's Beiträge*, **42**, 310.

Stübel, H. 1923. Die Ursache der Doppelbrechung der quergestreifter Muskelfasern. *Arch. f. d. ges. Physiol.*, **201**, 629.

Thorel, Ch. 1915. Pathologie der Kreislauforganen der Menschen. *4th. Ergeb. d. allge. Patbol. u. patbol. Anat.*, **17**, 90.

Warthin, A. S. 1925. The myocardial lesions of diphtheria. *J. Inf. Dis.*, **35**, 32.

Wegelin, C. 1913. Ueber alimentäre Herzmuskelverfettung. *Berl. klin. Woch.*, **2**, 2125 and 2190.

Werner, M. 1910. Besteht die Herzmuskulatur der Säugetiere aus allseits scharfbegrenzten Zellen oder nicht? *Arch. f. mikr. Anat.*, **75**, 101.

Witte, L. 1919. Histogenesis of the heart muscle of the pig in relation to the appearance and development of the intercalated discs. *Am. J. Anat.*, **25**, 333.

Zimmermann, K. W. 1910. Ueber den Bau der Herzmuskulatur. *Arch. f. mikr. Anat.*, **75**, 40.

Section XXIV

THE SPECIALIZED SYSTEMS OF THE HEART

CONTENTS

Section XXIV

		PAGE
I.	Technical Preparation.	856
II.	Distribution.	857
III.	Specific Features of Purkinje Cells	860
IV.	Cells of Embryonic Appearance .	862
V.	Purkinje Cells of "Adult" Type.	871
VI.	Longitudinal Segmentation or Cleavage of Purkinje Cells .	879
VII.	Transitional Types of Cell	881
VIII.	General Arrangement of Cells in Purkinje System.	882
	Summary.	882
IX.	Evidence of Pathology in Purkinje System .	883
X.	Bibliography.	884

Section XXIV

THE SPECIALIZED SYSTEMS OF THE HEART

T. WINGATE TODD

THE conducting system of the heart, or the Purkinje system as it is often and better named, consists of a network of cells of very special character permeating the myocardium and assumed, in the light of present-day knowledge, to carry the cardiac impulse. It is outside our immediate purpose to engage in a discussion of the physiological evidence for this view which is not without discrepancies and may require some modification before it truly expresses the facts.

Popular conception depicts the system as originating in the sinus node in the crista terminalis close to the orifice of the superior vena cava and thence passing by uncertain and largely theoretical connections to the atrio-ventricular junction where, beneath the septal cusp of the tricuspid valve, the tissue forms again in the atrio-ventricular node and from this point continues on the septum as two strands, one in the right and one in the left ventricle, to lose themselves in ramifications on the ventricular walls as the cells originally described by Purkinje.

This description is one of the most garbled perversions of fact in modern science, salted with a tiny pinch of truth to give it the tang of verity. Such truth as there is in it serves but to mislead and our task in the succeeding pages will be the substitution of a real picture for the false, recalling to serious attention the original paper by Stanley Kent, ignored during thirty years with a truly amazing persistence, but in which, nevertheless, every salient and important fact now known regarding the Purkinje system is already described. In brief, it may be stated at once that the Purkinje system is a ramification extending throughout the heart substance from the entry of the great veins to the terminal expansions in the ventricular walls, passing through the atrio-ventricular junction in diverse manner by several alternate paths. Of this plexiform network, through the hazard of chance, certain particular areas have received special attention, namely the sino-auricular node, the atrio-ventricular node and the so-called bundle of His. On these alone has attention generally been fixed, to the exclusion of other parts of the system, until popular assumption has endowed these areas with properties which are not their sole perquisite but characterize the system in its entirety. The areas mentioned are no different in structure from the remainder of the Purkinje ramifications.

It is not difficult to open the heart so as to conserve the main parts of the Purkinje system. A method for doing this was described long ago by Oppenheimer (1912) and this method still holds good but it must be

observed that Oppenheimer had in mind the display simply of the atrio-ventricular bundle and its node and did not consider the alternative paths from atrium to ventricle already mentioned and several times reiterated by Kent. Further, while preservation of the entire heart as suggested by Oppenheimer permits of a routine examination, at leisure, of the bundle and its connections, together with other parts of the system which have received but scant attention, adequate study of the cytology demands that very small sections be removed at once while the heart is still warm, and subjected immediately to fixing reagents. For the human hearts upon which this study is based we are indebted to Doctors E. P. Carter, R. W. Scott, H. Feil, H. M. Korns and A. M. Wedd and to each of these gentlemen I would formally acknowledge the opportunity they have given us at considerable personal cost in time and energy.

In 1892, A. F. Stanley Kent described, among other features, the bundle which, receiving attention the following year from W. His, Jr. (1893), has come to be known as the atrio-ventricular bundle of His. In 1906 Tawara found the atrio-ventricular node and the next year Keith and Flack discovered that there is another mass of tissue similar in structure, the sino-auricular node, in the crista terminalis, near the orifice of the superior vena cava, a site already identified by Wenckebach (1906) as containing fibers of significance which form a connection between the musculature of the great veins and that of the auricle.

For a few years after 1906 no great attention was paid to these anatomical characters but about 1909 the literature on the problem suddenly began to increase and for nearly a decade there were very active investigation and discussion regarding the functional characteristics of the system. Most observers appear to have started out with the assumption that the two nodes are the fundamental morphological features through which the cardiac impulse is regulated. With the established and evident fact of a progressive diminution in automaticity from sinus region (crista terminalis) to ventricle and with the progress of negativity there can be no quarrel but it is difficult to understand why certain portions of the Purkinje network, differing not at all in morphological characters from the rest of the extensive network, have been singled out and emphasized by physiologists in spite of equivocal and contradictory results and in the face of much contrary pathological evidence. It would almost appear as if experimental observers had read into the anatomical reports an interpretation against which they were warned in the very beginning by Stanley Kent, and had assumed that the "nodes" were isolated masses of very special tissue which could be investigated as discrete entities rather than parts of a network. For a summary of the many and ingenious experiments, often indeed, in technique, so injurious to the heart muscle that it is presumption to hope for unequivocal results from them, the article by Eyster and Meek (1921)

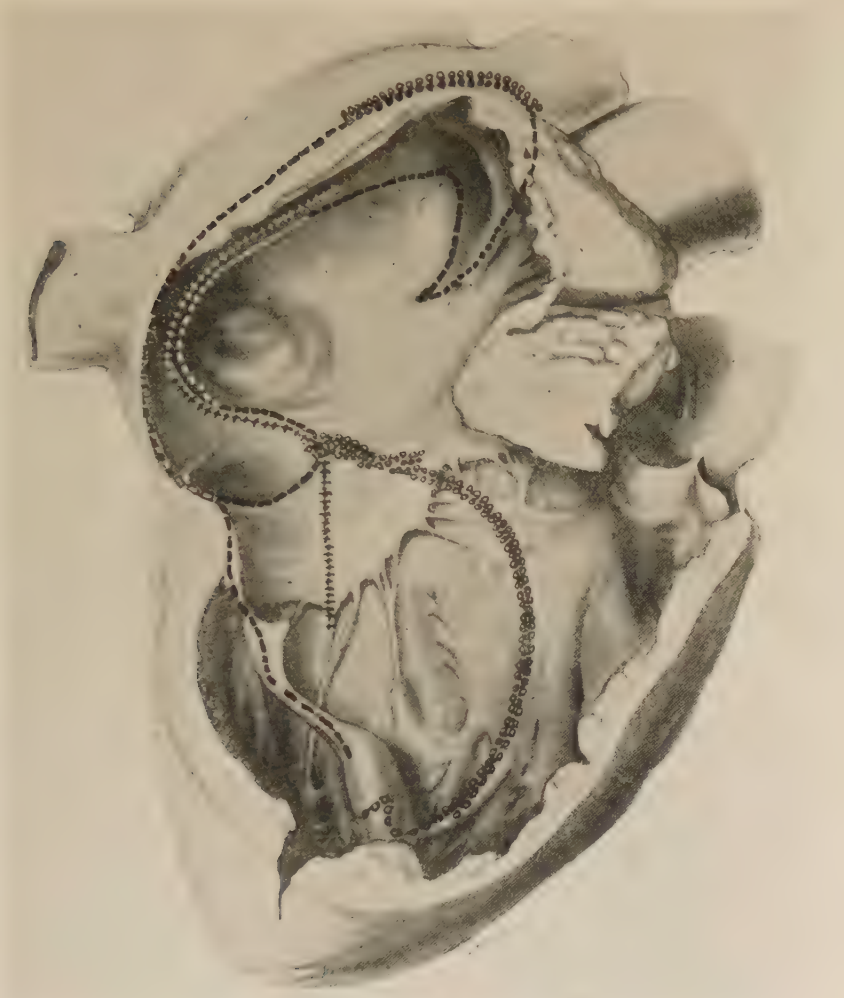


FIG. 367.—Diagram of human heart, constructed to show the relative positions of Purkinje systems in fish, reptile and mammal. We find the system in Man and dog occupying all the positions indicated and ramifying beyond those shown. The broken line is the site of specialized tissue in the fish; crosses indicate the site in the crocodile; small circles depict the course as usually understood in the mammal; the sino-auricular node is near the left venous valve in the crocodile and near the right venous valve in the mammal. (Ivy Mackenzie, 1913.)

should be consulted. In the second decade of this century there was a marked diminution in number of contributions to the subject and we are unlikely to see a revival of interest until the anatomical features have been cleared up. For a considerable number of years this investigation has been largely in the hands of workers of limited experience and background so that there is little to be gleaned from recent publications on the anatomical aspect. Chief among the deterrents are the peculiar staining reactions to which therefore we must devote some attention.

Physiologically one may summarize our knowledge by saying that all cardiac impulses whether normal or abnormal will be found to originate somewhere along the Purkinje system. But since the ramifications of this system permeate the entire heart this fact is of no striking significance. It is however well to mention that even auricular fibrillation is probably no exception to the rule. Further, experimental evidence, necessarily negative, indicates that the regions possessing nodal tissue alone show automaticity in the intact normal heart. Apart from its negative character this evidence is equivocal since there is no histological distinction between nodal tissue itself and the ramifications of nodal tissue. Experimentally conduction is rather diffuse and certainly, in the mammalian ventricle, does not seem to be a simple wave propagated along a single path. The diffuseness of conduction is borne out by the examination of pathological hearts.

The clue to the main ramifications of the system was given by Ivy Mackenzie (1913), in a study based on comparative anatomy. Mackenzie's diagram is reproduced as Fig. 367, a diagram of the human heart with the right atrial and ventricular walls removed to show the relative positions of specialized (Purkinje) tissue in fish, reptile and mammalian hearts. To a certain extent this diagram has sunk into the consciousness of other workers but its full significance has scarcely been grasped. The mammalian heart, including that of Man, possesses all these connections together with many ramifications naturally omitted by Mackenzie. It is the basis from which the present description starts.

Koch (1922) gives a good and fairly comprehensive account of work done up to 1922 but adds little new information regarding the Purkinje system. His figure (Koch Tafel XIII) of the system and its ramifications is much less useful than Mackenzie's because it is quite incomplete and takes no account even of the comparative researches mentioned in the text.

I. TECHNICAL PREPARATION

Since the Purkinje system lies immediately beneath the endocardium, fixation in situ can be attained provided the heart is properly opened and the entire endocardial surface exposed to the fixing fluid. For cytological

studies this does not give proper fixation and specimens must be obtained while the heart is yet warm. With the general distribution of the Purkinje system in mind small blocks of tissue about 5 mm. square and 10 mm. long (smaller for osmic fixation) may be removed by a sharp knife. Presence of Purkinje leashes can readily be ascertained by observation of the cut end, for in fresh tissue the Purkinje fibers contract more than the cardiac fibers and dimpling is seen on the fresh cut surface.

For general purposes Bouin's fluid or 5 per cent trichloracetic acid in water are good fixatives but for cytology an osmic fixing agent like Hermann's or Flemming's solution is the best.

If Bouin or trichloracetic acid is used, hardening in 70 per cent alcohol, to which a few drops of tincture of iodine has been added as a mordant, makes a good preparation and before embedding in paraffin the tissue may be stained en bloc in borax carmine.

Two reasons for the great diversity of result in histological examination are: (1) the utter impossibility of examining the entire ramifications of the system and (2) the very different pictures shown by various staining methods. Eosine is a muddy counterstain for Purkinje fibers and should never be used. With hematoxylin, congo red gives a much better counterstain, though even this is of much less general usefulness than the methods which follow:

For the cell structure Mallory's method modified by Sabin (Mall, 1902) is the best. Iron hematoxylin gives a very different picture of the Purkinje fiber in that it emphasizes a transverse striation of which Mallory's method makes little record.

Sections stained by safranine or fuchsin and light green give beautiful preparations of the cement or junctional lines (intercalated discs) and differentiate between the myofibrils and their modified substance in the disc itself. The intracytoplasmic discs disposed in terraced arrangement, as described by Jordan (e.g. 1917) and his colleagues in various papers are also well demonstrated by this method.

II. DISTRIBUTION

Although Purkinje's original description is limited to the elements in the terminal ventricular expansions of the system and although this definition has been frequently followed by other observers, it is convenient to describe the elements of the entire system as Purkinje cells, and the chains of cells as Purkinje fibers, for there is no difference between the elements in the several parts of the system. We find Purkinje cells throughout the human heart from the orifices of the venae cavae and coronary sinus to the ventricular expansions. While the cells are found in leashes of fibers scattered throughout the cardiac substance immediately beneath the

endocardium, they are concentrated into relatively small compass at the atrioventricular junction. This however does not mean that there is only a single bundle of Purkinje fibers passing between the right atrium and the ventricles. Stanley Kent (1893), in his first description, warned other observers that the distribution of Purkinje fibers at this site is by no means invariable but this warning has usually passed unheeded: indeed many authors have failed to confirm Kent's statement. This failure has resulted in one of the many pitfalls awaiting the experimental worker for there is no doubt that Kent was right in his conclusion. Negative evidence on the Purkinje system means inadequate observation due to the technical difficulties of the problem.

The information upon which the following account is based was published in 1919 by Professor Van der Stricht and the present author. It was confirmed and extended during the past decade by the latter.

We shall not pause to consider the profuse network of nerve fibers surrounding and intermingling with the Purkinje system nor the nerve cells which are to be found at frequent intervals throughout its course. They have been studied by several workers since Wilson (1909) first made a special and intensive investigation. Jean Meiklejohn (1914) gave a good summary of previous work and a record of her own researches in 1914. In ungulates and rodents the nerves are easily demonstrated and Meiklejohn got good preparations in the monkey (Callithrix). The nerves of Carnivora and man have been disappointing: the technique used apparently does not readily pick out the nerves in these forms. It cannot, however, be that there is any real difference in mammals and doubtless the nerves in Carnivora and in man will be eventually adequately demonstrated.

We shall not devote attention to the adjacent lymphatic spaces described particularly in the ungulate heart (Lhamon, 1912). Rénon (1914) has described elastic fibers in abundance in the bundle of His and in relation to the sino-auricular node. While these certainly occur it is necessary to avoid confusing them with the great masses of elastic fibers belonging to the endocardium. The heart consists embryologically of two parts, an inner arterial component and a surrounding myocardial mantle (Ingalls, '07,' 20). The arterial component consists of endocardium with smooth muscular fibers most numerous in the right atrium especially near the orifices of the great veins but also in lesser numbers, sometimes as isolated scattered fibers near the middle of the thickness of the endocardium; sometimes as bundles or even as a continuous layer, over the entire ventricular area. They are least numerous over the right ventricular branch as it lies upon the septal wall (Fig. 368).

Immediately beneath the endocardium and superficial to the Purkinje fibers which of course belong to the myocardial mantle are numerous elastic fibers forming three membranes successively of thicker fibers as we pass

from endocardium outwards (Fig. 369). In areas where the endocardium is thinnest, the elastic laminae dwindle in number and in size of elements.

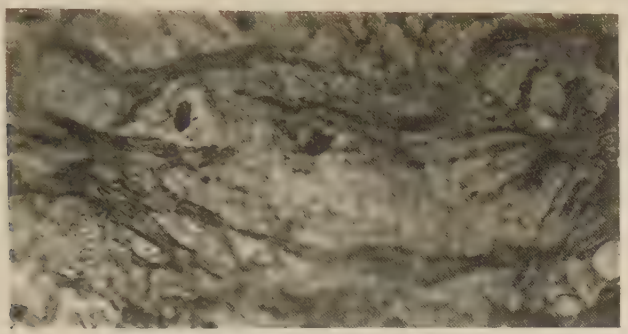


FIG. 368.—Smooth muscle fibers from endocardium over right atrioventricular junction. Age thirty-six years. 476 diameters. (Bouin; Mallory.)

FIG. 369.—Elastic fibers of endocardium. Septal wall, right atrium. Age thirty-six years. 476 diameters. The fibers increase in size with depth from surface. (Hermann; iron hematoxylin; congo red.)

But clearly these fenestrated elastic laminae belong to the endocardium and can in no sense be invoked in the manner Rénon has described them as necessary to the function of the atrio-ventricular bundle.

We shall then confine our attention to the Purkinje fibers themselves.

The Purkinje system forms a network throughout the heart just as fully permeating the atrium as it does the ventricles. The model by De Witt (1909) of the ramifications in the ventricle may be taken as an illustration also of the far-reaching character of Purkinje fibers in the atrium. There is no part of the right atrium which is devoid of Purkinje fibers. I cannot speak with this certainty for the left atrium for we have never concentrated our study upon this part of the organ. It is not possible to pick out special leashes of fibers in the atrium similar to the bundle of His any more than it is possible to do so in the so-called terminal arborizations in the ventricles. The concentration of fibers at the atrioventricular junction is a natural result of the reduction of musculature at this site. The sino-auricular node and the so-called upper or atrial portion of the atrioventricular node are merely atrial masses of Purkinje fibers easily located and demonstrated.

For the figures which illustrate this article I have chosen microphotographs from two hearts only, the one from a white male of thirty-six years dead of pneumonia, the other from a colored male of forty-two years whose death occurred from syphilitic myocarditis. The thirty-six year heart weighed 240 gm. and the forty-two year organ weighed 770 gm. It must not be supposed that a pathological heart necessarily presents abnormal or diseased Purkinje cells throughout the system. Indeed some of our finest preparations of normal Purkinje cells are from the atrial septum and sinus node region of pathological hearts from patients of over sixty years.

The material upon which the present study is based consists of human hearts of all periods in life from birth to old age; hearts of both sexes, of white and colored patients; some normal so far as we could judge them, others pathological with partial or complete heart block, or with no heart block. The series includes syphilis, diphtheria and tumors and has been increasing in numbers for ten years. In addition we have examined a considerable number of dog hearts of various ages. All hearts have been obtained and fixation carried out while still warm. The technical methods used and the pieces chosen are fully described in our earlier monograph (Van der Stricht and Todd, 1919).

The figures are almost constantly reproduced at a magnification of 476 diameters so that dimensional differences may be more readily appreciated.

III. SPECIFIC FEATURES OF PURKINJE CELLS

Obermeier (1866) distinguished three forms of Purkinje cells:

1. Spheroidal cells with a finely striated cortex and an abundant protoplasm. They contain two or three nuclei.

2. Cells more elongated, with a cortex thicker and more obviously striated and with less abundant protoplasm. Their nuclei are fewer in number.

3. Other cells even more elongated, more slender and with a striated cortex occupying almost the entire thickness of the cell body. These cells are gradually continuous with ordinary cardiac fibers.

Many authors have described the presence of glycogen droplets in Purkinje cells and Mönckeberg (1908) attaches great importance to their occurrence. He notes in the child's heart the continuity of the left bundle branch with Purkinje fibers very similar in appearance to neighboring cardiac fibers. As cardiac cells contain no glycogen Mönckeberg suggests that the presence of glycogen affords a certain criterion for the discernment of a Purkinje cell from an ordinary cardiac muscle cell. The fact that Mönckeberg lays emphasis upon the child's heart is important. We find Purkinje fibers in the young heart much more difficult to distinguish from cardiac fibers than in the adult.

Marceau (1902), working upon the sheep heart, described the Purkinje cell as having four layers:

1. A central zone absolutely unstained, enclosing the nuclei, possibly an artefact resulting from shrinkage induced by reagents, a suggestion already made by Hoyer (1899).

2. A granular layer surrounding the foregoing, dotted throughout by rounded granules of variable size and staining intensely with iron hematoxylin. The ability of this stain to pick out these granules is especially worthy of notice because, in consequence, Purkinje cells stained with iron hematoxylin present an appearance markedly different from that resulting from the use of Mallory's stain. With iron hematoxylin the cells much more closely resemble cardiac cells, whereas Mallory's stain emphasizes the difference.

3. A stratum uniformly colored pale grey in which very fine isolated longitudinal striations may be distinguished but in which transverse striation is scarcely perceptible.

4. A striated cortex in which the striae run circularly, obliquely, or longitudinally.

It is of no real consequence what mammalian heart be studied. The Purkinje cells present the same characters in all, but the distinguishing features are more easily discerned in some animals than in others. Hence the frequency with which the ungulate heart forms the object of investigation. In dog, Purkinje elements are not so definitely distinguishable from true cardiac cells as in the sheep, a fact also noted by Tawara (1906).

In the human heart we have described two well-defined types of Purkinje cells with stages intermediate between them and also others, transitional between Purkinje cells and true cardiac cells. To distinguish these two main types we have defined them as:

1. Cells of embryological aspect.

2. Cells of adult type.

It was by no means intended that this definition should imply that we considered the cells of embryological aspect to be cells in an embryonic stage. It is simply a convenient way of calling to mind in a word the appearance of the cell. Nevertheless succeeding workers have misunderstood our

meaning and either have sought to elaborate the idea of Purkinje cells being embryonic cardiac cells (Retzer, 1920), or have been at great pains to refute the suggestion (Cady, 1921; Tufts, 1921).

IV. CELLS OF EMBRYONIC APPEARANCE

These cells are bulky, polyhedral, polygonal or even prismatic in section, the longitudinal diameter being a little greater than the transverse. Towards the center of the protoplasm they show two, three, four or even five nuclei (Figs. 370 and 371) which are relatively small, oval, of equal size, often joined together but otherwise tending to be shifted along or near to the axis of the cell.

The cytoplasm presents a central perinuclear granular area and a cortical fibrillar zone. The main substance of the central area is pale and

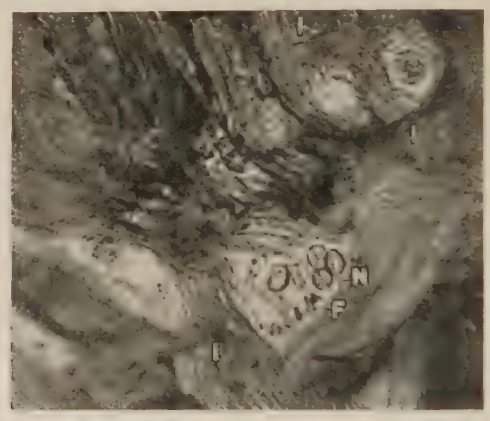


FIG. 370.—Purkinje cells, posterior papillary muscle left ventricle. Age thirty-six years. 476 diameters. F, fat granules; I, intercalated disc; N, four nuclei. (Hermann; Mallory.)

non-staining, perfectly homogeneous in some preparations but exhibiting in others a delicate meshwork enclosing a pale liquid. Immediately surrounding the nuclei is a clear area described by Schockaert (1909), which contains absolutely no mitochondria and is of variable size in different preparations.

There do occur in the axial region of some Purkinje cells of this type one or more compact almost homogeneous bodies staining with congo red or light green. In transverse sections these look like an attraction sphere but in longitudinal sections they often form a kind of interrupted strand (Fig. 372).

This granular area contains many chondriomites, the axis of which approximates that of the cell (Fig. 372). There are also coarser pigmentary

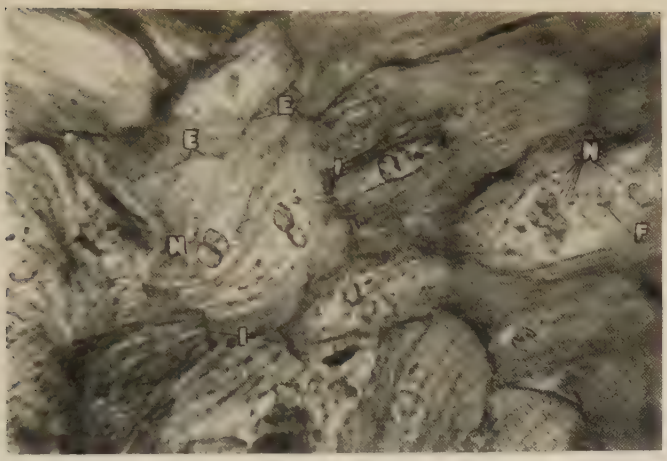


FIG. 371.—Purkinje cells, posterior papillary muscle left ventricle. Age thirty-six years. 476 diameters. E, endomysium; F, fat granules; I, intercalated disc. N, multiple nuclei, four in one cell, five in the other. The cell with four nuclei has a fibrillar differentiation passing right through the axial sarcoplasm (Cf. Figs. 373, 374). (Hermann; iron hematoxylin; congo red.)

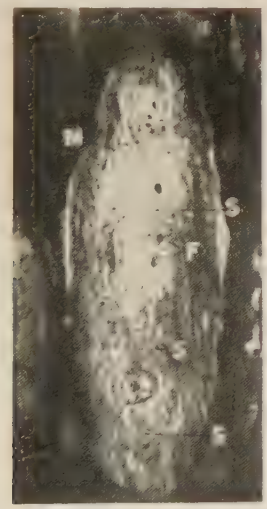


FIG. 372.—Purkinje cell from left posterior ventricular branch of the bundle. Age forty-two years. 476 diameters. Note perinuclear granular and cortical fibrillar areas. F, fat granules; M, mitochondria; S, axial interrupted cytoplasmic strand. (Hermann; iron hematoxylin; congo red.)

and fatty granules. In addition there may be seen, though not invariably, fibrillae, transversely striated, either isolated or grouped into very fine bundles.

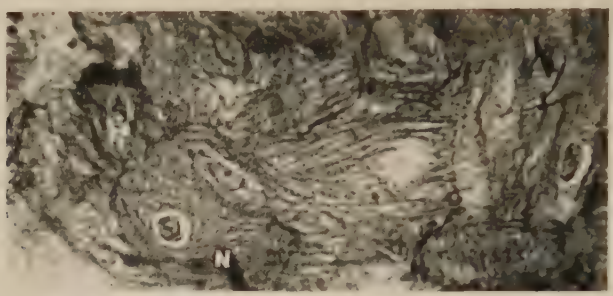


FIG. 373.—Purkinje cells from atrioventricular node. Age thirty-six years. 476 diameters. N, cell with two nuclei separated by axial fibrillar mass (Cf. Figs. 371, 374). (Hermann; Mallory.)

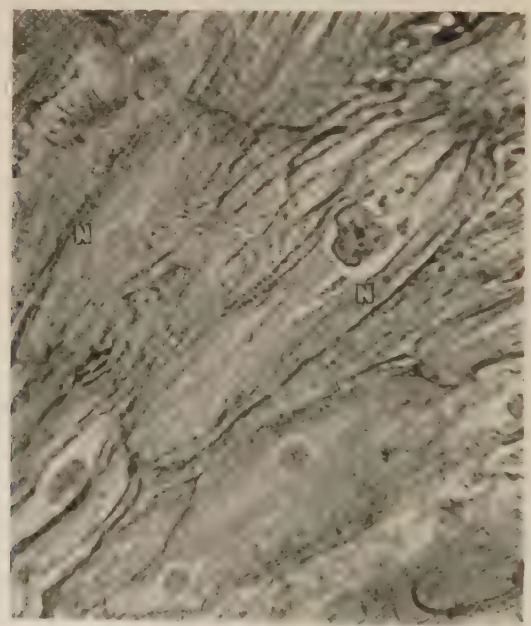


FIG. 374.—Purkinje cells from moderator band right ventricle. Age thirty-six years. 476 diameters. NN, cell with two nuclei separated by axial fibrillar mass (Cf. Figs. 371, 373). (Bouin; Mallory.)

The cortical zone has also a granular sarcoplasm with abundant mitochondria but is plainly fibrillar, the fibrillae being similar to those already described but far more numerous. This zone may be very thin or may

encroach considerably upon the granular area. Indeed in certain cells, the nuclei of which are laterally placed, the fibrillae even differentiate in the axial sarcoplasm and thus subdivide the cell into two (Figs. 371, 373, 374). This double character becomes more obvious in the second or "adult" type of Purkinje cell.

I have mentioned the transverse striation of the fibrillae in these "embryonic" Purkinje cells. The fact that this feature is as characteristic of Purkinje cells as it is of cardiac fibers would suggest that there may be some difficulty in making the distinction. It must therefore be emphatically stated that between these two elements there is a profound difference. Figure 375 illustrates the appearance of true cardiac cells with their oblong

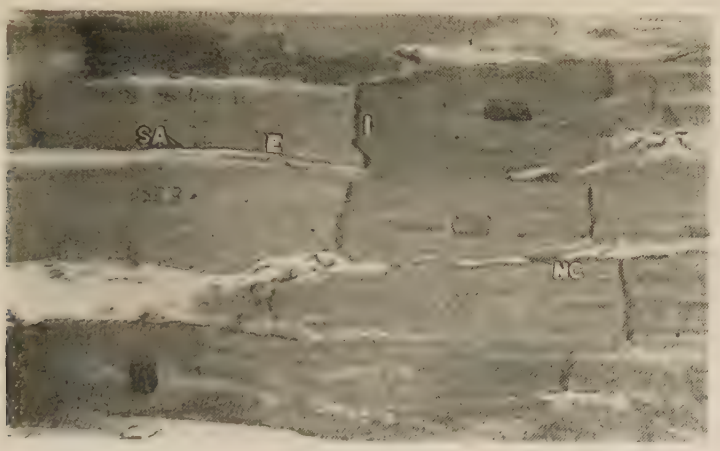


FIG. 375.—Cardiac muscle cells from posterior papillary muscle of left ventricle. Age thirty-six years. 476 diameters. E, endomysium; I, intercalated disc; NC, nucleus of connective tissue cell; SA, sarcolemma. (Hermann; Mallory.)

nucleus, thick limiting discs, profuse and heavily staining fibrillae, regularly disposed and very obviously transversely striated. This preparation was fixed with Hermann's fluid and stained by Mallory's method so that it may be compared in detail with the Purkinje preparations. The difference between "embryonic" Purkinje cells and cardiac cells lies not only in size and form but also in number of nuclei, quantity of sarcoplasm and fibrillae and still more in transverse striation which is relatively much less marked in the Purkinje cell. If these distinctions be borne in mind it is not difficult to pick out intermediate or transitional forms of which the most characteristic is to be described later as an "adult" Purkinje cell.

The fibrillae of the "embryonic" Purkinje cell are not uniform in arrangement but present three types of intracellular orientation.

1. Cells of which the fibrillae are parallel with each other and more or less with the cell axis. (Figs. 370 to 372.)

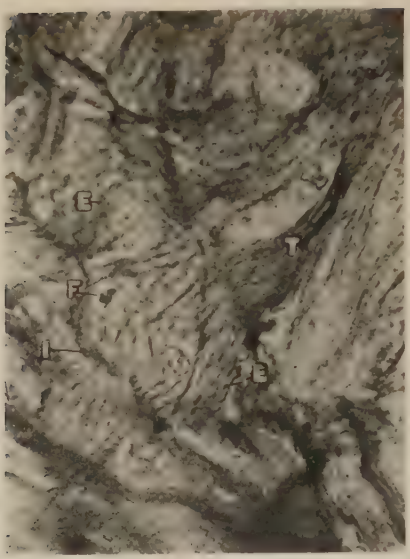
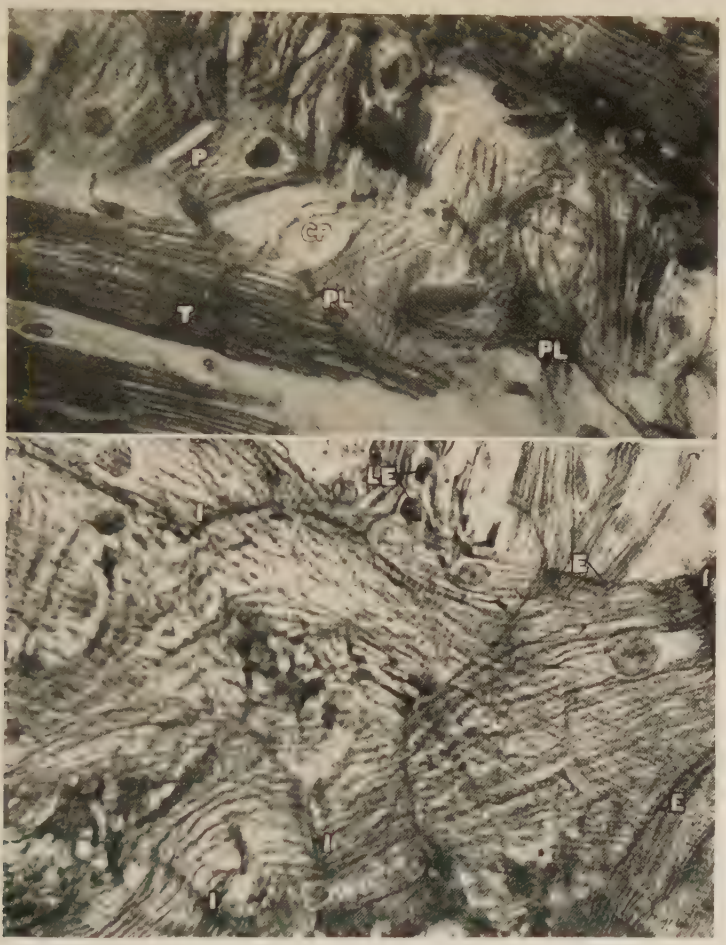


FIG. 376.—Purkinje cells from the atrioventricular node. Age thirty-six years. 476 diameters. E, endomysium; F, fat granule; I, intercalated disc; T, transitional cell (i.e. intermediate in appearance between Purkinje and true cardiac types). (Hermann; Mallory.)

FIG. 377.—Purkinje cells from moderator band right ventricle. Age thirty-six years. 476 diameters. Note intercrossing and plexiform fibrillae and the transverse striation of the fibrillae. E, endomysium; F, fat granules; I, intercalated disc; NC, nucleus of connective tissue cell. (Hermann; safranine; light green.)

2. Cells with intercrossing fibrillae. (Figs. 373, 374, 376, 377.)

3. Cells with plexiform fibrillae. These fibrils intercross in many directions to form an intracellular plexus (Figs. 377 to 380).



378

379

FIG. 378.—Purkinje cells with plexiform fibrillae and transitional cells from moderator band right ventricle. Age thirty-six years. 476 diameters. CP, capillary; P, Purkinje cells; PL, Purkinje cells with plexiform fibrillae; T, transitonal cell. (Bouin; iron hematoxylin; congo red.)

FIG. 379.—Purkinje cells with plexiform fibrillae from moderator band right ventricle. Age thirty-six years. 476 diameters. Note double character of intercalated discs. E, endomysium; I, intercalated disc; LE, leucocyte. (Bouin; Mallory.)

Now it is true that there is no comparable intercrossing or plexiform arrangement of the fibrillae in the true cardiac cells. These, however, branch

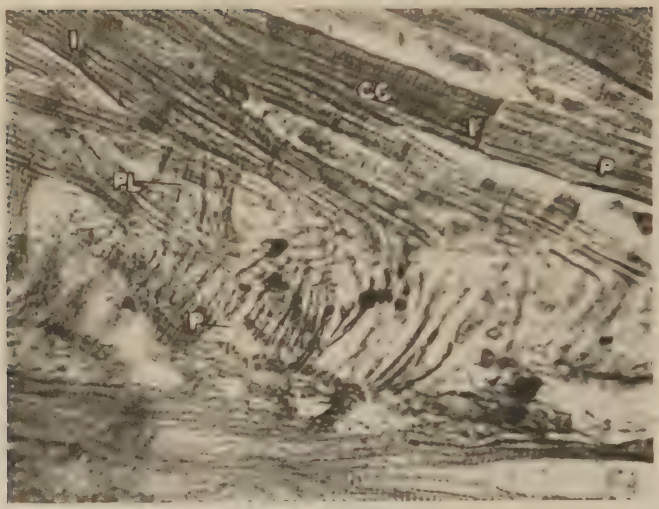


FIG. 380.—Purkinje cells with plexiform fibrillae and transitional cells, moderator band, right ventricle. Age thirty-six years. 476 diameters. CC, cardiac cell; I, intercalated disc; P, Purkinje cell; PL, Purkinje cell with plexiform fibrillae. Bouin; Mallory.)

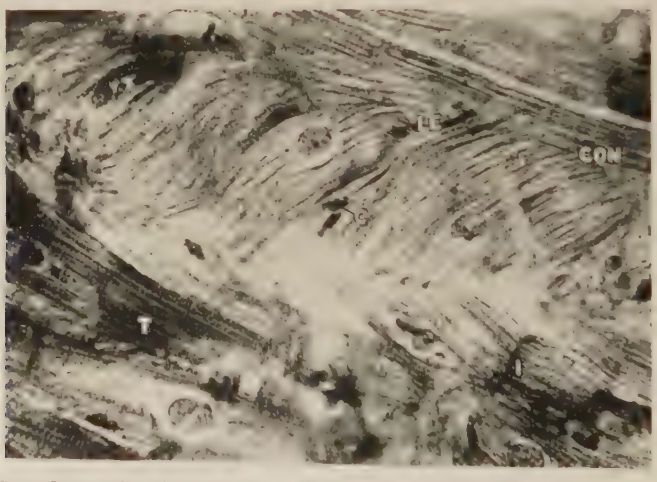
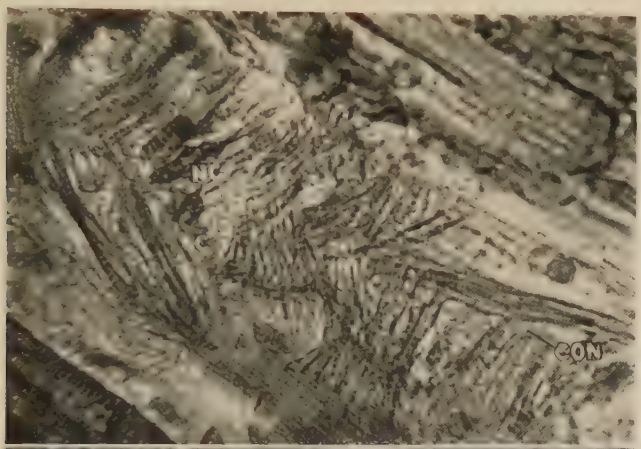


FIG. 381.—Intercalated or association Purkinje cells from moderator band right ventricle. Age thirty-six years. 476 diameters. C, connective tissue nucleus; CON, continuity of fibrillae from one cell to another; I, intercalated disc; LE, leucocyte; T, transitional cell. (Bouin; Mallory.)

and anastomose (Fig. 375), a feature also exhibited by the Purkinje cells (Fig. 377). Of this branching and anastomosis the intercrossing of fibrillae is a part.

382



383

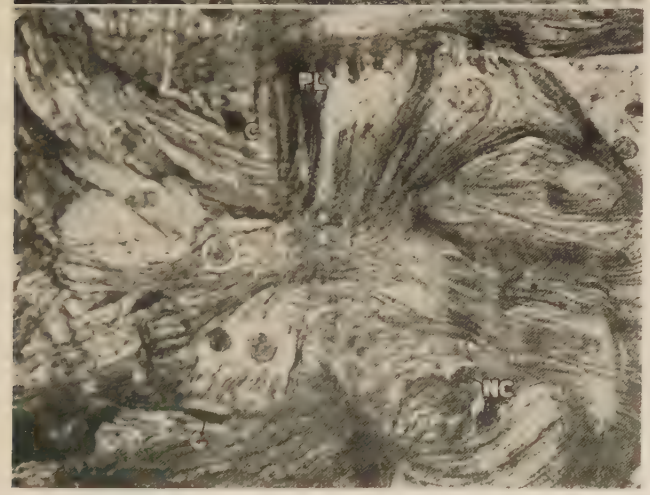


FIG. 382.—Association Purkinje cells with plexiform fibrillae, moderator band right ventricle. Age thirty-six years. 476 diameters. C, connective tissue; CON, continuity of fibrillae from one cell to another; NC, nucleus of connective tissue cell. (Bouin; Mallory.)

FIG. 383.—Stellate grouping of Purkinje cells from moderator band, right ventricle. Age thirty-six years. 476 diameters. C, connective tissue; PL, Purkinje cell with plexiform fibrillae; NC, nucleus of connective tissue cell. (Bouin; iron hematoxylin; congo red.)

We must therefore describe the intercalated and association type of Purkinje cell.

Intercalated or association cells are interposed either between two bundles of Purkinje fibers the axes of which are parallel, or between a

bundle of Purkinje fibers and a bundle of cardiac fibers. As the intercalated cells are irregularly polyhedral, a little longer than they are broad (Figs. 378, 380, 381), they belong to the "embryonic" type of Purkinje cell. Their axes are either at right angles or oblique to the axis of the neighboring bundles. The simplest possess parallel fibrillae (Fig. 381), others intercrossing or plexiform fibrillae, (Figs. 378, 379, 382). The fibrillae pass uninterruptedly into the cytoplasm of the cells which they connect and in the interior of which they become continuous with the longitudinal fibrillae (Fig. 381).

Sometimes these intercalated cells form veritable networks in the spaces of which there is a vascular connective tissue with, according to Tawara (1906), small nerves. We have not made special preparations for study of the nerves. Sometimes intercalated discs delimit the apposed ends of these cells. Sometimes the discs are absent and the network is then a true syncytium.

Tawara (1906) also observed this plexiform arrangement and described it rather roughly. He, however, thought it characteristic of the area described by him as the Knoten, and this is really the morphological basis for the conception of an auriculo-ventricular node. We find the plexuses very numerous through the Purkinje system, from the region of the great veins to the terminal ventricular expansion, and I can therefore see no special anatomical reason for believing in a Knoten at all. Mönckeberg (1908) and De Witt (1909) also observed the plexiform appearances.

We have found a stellate grouping (Fig. 383) especially in the trabecular tissue of the left ventricle.

In summary we may conclude that, throughout the Purkinje system there are to be found bulky cells, a little longer than broad, and polyhedral in outline, some possessing fibrillae parallel with the long axis of the cell, others showing intercrossing or even plexiform fibrillae; and that certain of these cells act as intercalated or association elements between neighboring bundles of cells, whether of Purkinje or true cardiac type. Cells of this variety we have designated as "embryonic" in type.

In general such cells exhibit a very precise delimitation. Along the lateral surfaces of the polyhedral cell is a very fine, often vascular, connective tissue, a sort of endomysium which separates it from neighboring cells, stained blue by Mallory's method or green with light green (Figs. 370, 373, 374, 377, 379, 386). Other surfaces or ends of the polyhedron are delimited from the cytoplasm of neighboring cells by intercalated discs absolutely identical with those of the true cardiac cells and stained red by Mallory or safranine. This well-marked delimitation is however lacking at the extremities of the intercalated cells (Figs. 378, 380 to 382) and also at the extremities of certain of the stellate groupings (Fig. 383).

V. PURKINJE CELLS OF "ADULT" TYPE

Although cells of the "embryonic" variety are to be found throughout the Purkinje system, a great majority of Purkinje tissue is made up of

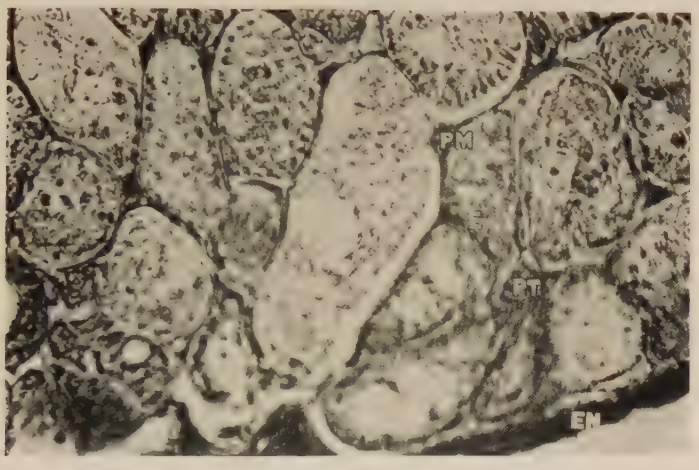


FIG. 384.—Purkinje cells from left posterior ventricular branch in the trabecular tissue. Age thirty-six years. 535 diameters. E, endomysium; EN, endocardium; PM, perimysium; PT, Purkinje mother-cell splitting into daughter-cells. (Hermann; safranin; light green.)

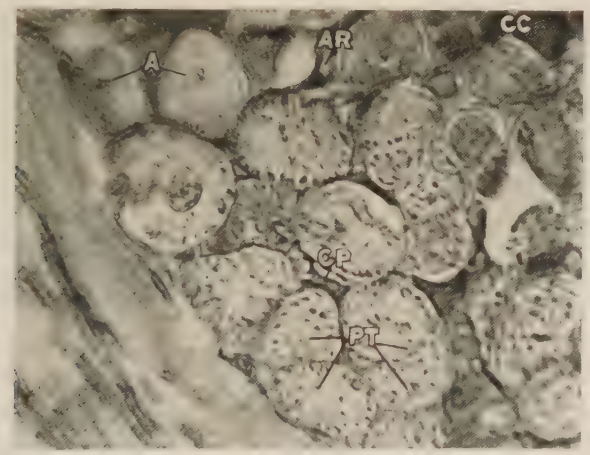


FIG. 385.—Purkinje cells from septal wall of right atrium. Age thirty-six years. 476 diameters. A, asymmetrical Purkinje daughter-cells; AR, artery; CC, cardiac cell; CP, capillary; PT, Purkinje mother-cell dividing. (Hermann; safranin; light green.)

"adult" cells. These are elongated, cylindrical cells, or through reciprocal pressure, prismatic or flattened. In transverse section they appear tessellated (Figs. 384 to 386).

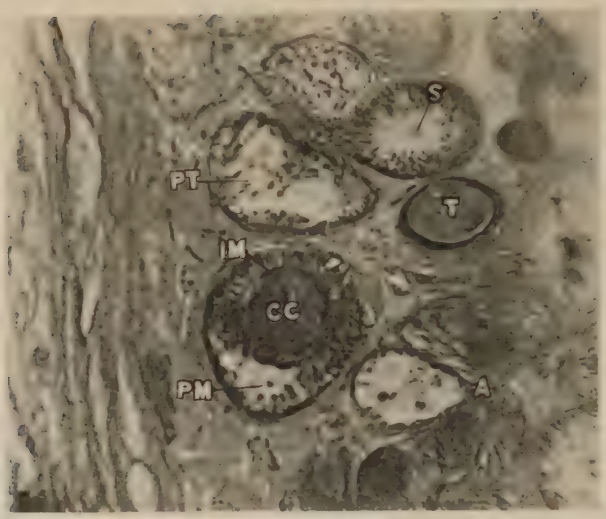


FIG. 386.—Purkinje cells from left posterior ventricular branch. Syphilitic myocarditis. Age forty-two years. 476 diameters. Note the largely increased connective tissue. A, asymmetrical Purkinje daughter-cells; CC, cardiac cell; IM, internal cytoplasmic membrane; PM, Purkinje mother-cell; PT, Purkinje mother-cell dividing; S, axial strand; T, transitional cell. (Hermann; Mallory.)

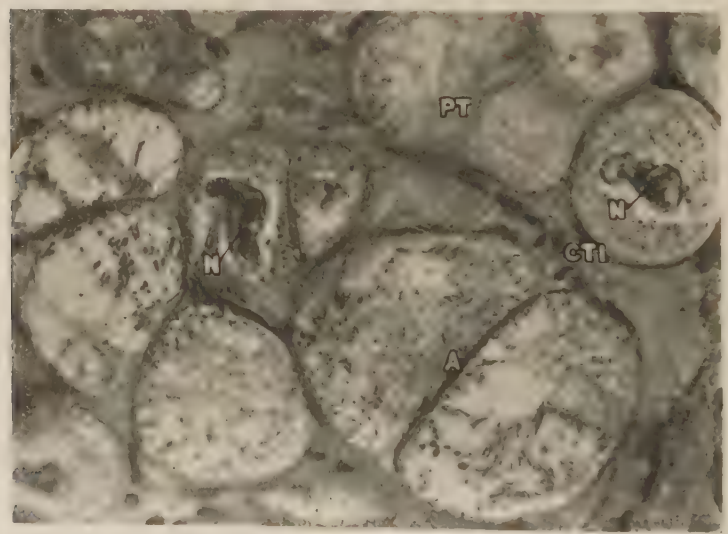


FIG. 387.—Purkinje cells from left posterior ventricular branch in the trabecular tissue. Age forty-two years. Syphilitic myocarditis. 476 diameters. A, Purkinje daughter-cells; CTI, infiltrated connective tissue; PT, Purkinje mother-cell dividing; N, very large Purkinje nucleus. (Hermann; iron hematoxylin; congo red.)

The "adult" cells are multinuclear like the "embryonic" type and the nuclei are round or oval, subequal in size and a little larger than those of the

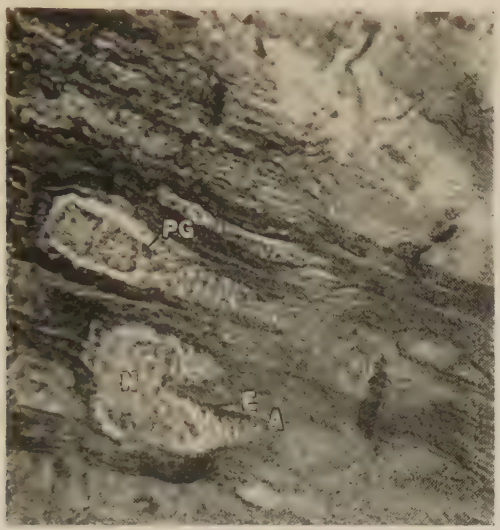


FIG. 388.—Purkinje cells from septal wall right atrium. Age forty-two years (trichloracetic acid; Mallory . 476 diameters. A, Purkinje daughter-cells; E, endomysium; PG, pigment granules; N, very large nucleus being subdivided by encroaching strand.

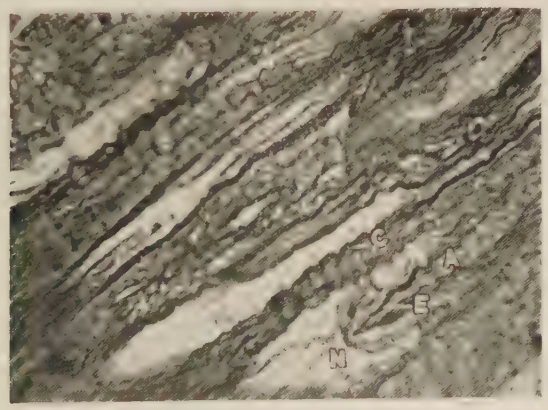


FIG. 389.—Purkinje cells from left posterior ventricular branch on septal wall. Age forty-two years. 476 diameters. A, Purkinje daughter-cells; C, connective tissue; E, endomysium; N, very large nucleus being subdivided by encroaching strand. (Bouin; Mallory.)

"embryonic" cell although occasionally one meets with very large nuclei (Figs. 387 to 389). As the cell approaches in its general appearance the true cardiac cell, so in like measure the nuclei more closely resemble those of the

cardiac cells in morphological characters (Fig. 390). It is difficult sometimes to estimate the number of nuclei in any single cell for they are apt to lie

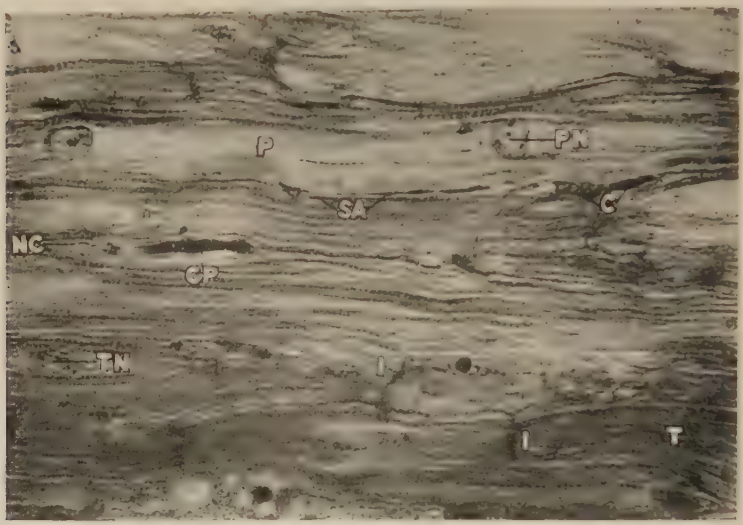


FIG. 390.—Purkinje cells approaching in general appearance to cardiac cells, from left posterior ventricular branch in trabecular tissue. Age thirty-six years. 476 diameters. C, connective tissue; CP, capillary filled with red cells; I, intercalated disc; NC, nucleus of Purkinje cells; P, Purkinje cell; T, transitional cell; TN, nucleus of transitional cell; SA, sarcolemma. (Hermann; Mallory.)

FIG. 391.—Purkinje cells from main left ventricular branch immediately below aortic cusp. Age thirty-six years. 476 diameters. Two short bulky cells, each with two nuclei. C, connective tissue; E, endomysium; F, fatty granules; I, intercalated disc; PT3, cell dividing into three daughter-cells. (Flemming; safranin; light green.)

quite distant from each other and are variously distributed round the cellular axis. This shifting is more marked than in the "embryonic" cells in both lateral and longitudinal directions (Fig. 392). Figures 391 to 393

show successive stages in the growth and elongation of Purkinje cells and in the shifting of nuclei. Figure 391 shows two rather short bulky cells each with two nuclei. The left cell has within it a septum of fibrillae coursing obliquely through the cytoplasm. Figure 392 shows a bulky but longer cell enclosing five nuclei with several septa of fibrillae partially subdividing the cell substance. Figure 393 shows a still longer cell with one group of two nuclei and a third nucleus at some distance.

392

393

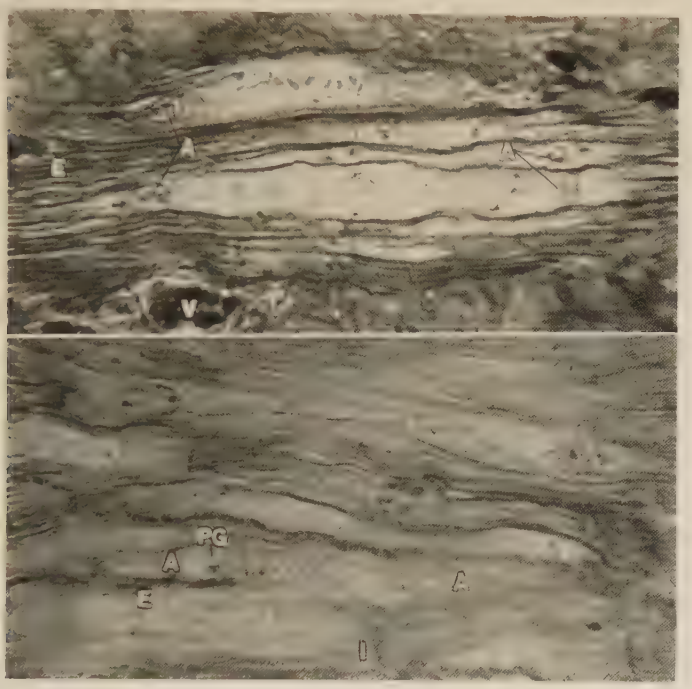


Fig. 392.—Purkinje cell from main left ventricular branch immediately below aortic cusp. Age thirty-six years. 476 diameters. A, bulky but larger cell with five nuclei, two at left and three at right; E, endomysium; V, vein. (Flemming; safranin; light green.)

Fig. 393.—Purkinje cell from main left ventricular branch immediately below aortic cusp. Age thirty-six years. 476 diameters. AA, longer cell with two nuclei together and one at a distance. Partially subdivided; E, endomysium; I, intercalated disc; PG, pigment granules. (Flemming; safranin; light green.)

The sarcoplasm is similar in all respects to that of the "embryonic" type of cell. It contains the same clear perinuclear layer, the same mitochondria, fatty and pigmentary granules and the same axial strand. Space does not permit a fuller description of the sarcoplasm. It will be found, however, in a former publication (Van der Stricht and Todd, 1919). There are also rare fibrillae in the outer part of the central area.

The cortical zone like that of the "embryonic" cell possesses much more numerous fibrillae. There may however be a more or less uniform distribution of fibrils throughout the entire substance of the cell: even certain "embryonic" cells are thus constructed. The fundamental character by which one easily distinguishes Purkinje cells of either atrium or ventricle from cardiac elements is the constant presence in much greater abundance of an interfibrillar sarcoplasm. This gives the cells a quite distinctive clearer, less compact appearance than the cardiac cells, a feature long ago insisted upon by Keith and Flack (1905) and others.

The Purkinje cell is typically of greater diameter than the cardiac cell. It is both longitudinally and transversely striated but the transverse striae are very much less pronounced than in true cardiac cells, though likewise observable, especially after staining with iron hematoxylin or with the use of polarized light (Johnstone and Wakefield, 1922).

The fibrillae of true cardiac cells and of Purkinje cells alike tend to unite in the midst of the cytoplasm into sarcostyles between which is a sarcoplasm more abundant than that between the fibrillae themselves. This arrangement is well marked in Purkinje cells but the form of the sarcostyle varies exceedingly from cell to cell and even within a single cell. Sarcostyles may occur as prisms, rods or hollow cylinders. The prisms and rods are compact bundles of fibrillae whereas the hollow cylinders are formed by a peripheral fibrillar sheath surrounding a sarcoplasmic axial portion (Fig. 384, large cell). Other sarcostyles are laminated and generally occur in the peripheral portion of the cell, appearing in transverse section as thick striae arranged radially, which may be simple and thick, or double and formed by two fine parallel streaks separated or united in part of their course (Fig. 384).

The fascicular arrangement of Purkinje fibers is not constant. Quite often there are areas in which the interfascicular tissue is not greater than the intrafascicular tissue and in such cases the fibers seem to be equidistant from each other.

The longitudinally arranged cells forming strands which are connected with neighboring similar strands by the "embryonic" intercalated cells, are usually of "adult" type.

Most authors agree that there is a connective tissue sheath with numerous elastic fibers surrounding several segments of the atrio-ventricular bundle. According to Fahr, the atrio-ventricular bundle in the human embryo contains a more abundant connective tissue than the cardiac muscle and thus one may distinguish it from the myocardium. Mall (1912) recognized the bundle in two embryos of 50 mm. length by the "more epithelial" character of its cells and was able to follow the differentiated bundle into two divisions: "one of which reaches the moderator band." Further on Mall adds: "It is thus seen that the atrio-ventricular bundle is

present in an embryo 8 mm. long and that as soon as the muscle of the rest of the atrial canal is broken down it becomes outlined by encircling spaces which are now formed."

Delimitation of the Purkinje cells of adult type is brought about like that of the "embryonic" cells by intercalated discs at the extremities and by an endomysial membrane on the lateral surfaces. In this precise delimitation, the Purkinje fibers, composed of cells placed end to end, resemble true cardiac fibers in which however the vascular interstitial connective tissue is much more abundant. If at first sight the lateral contours of the Purkinje cells are less distinct it is because the interstitial tissue between them is so reduced and because many cells are in a state of longitudinal cleavage, a phenomenon to be discussed later.

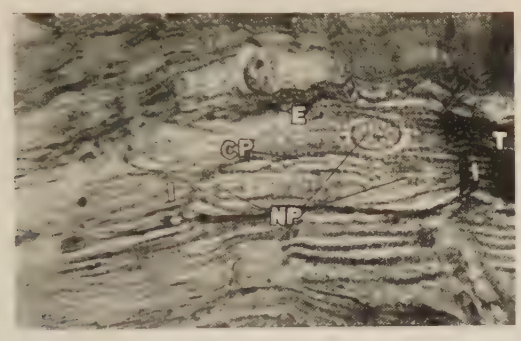


FIG. 394.—Purkinje cells from moderator band right ventricle. Age thirty-six years. 476 diameters. CP, capillary; E, endomysium; I, intercalated disc; T, transitional cell; NP, nuclei of one Purkinje cell. (Bouin; Mallory.)

The intercalated discs examined in longitudinal sections appear as transverse lines staining deeply with safranin or fuchsin. The disc is striated (Fig. 394) each stria corresponding to a thickening or more deeply colored disc of a fibril continuous across the junctional line. Not infrequently one sees double intercalated discs formed from two parallel bands united by finer fibrillae to form a species of intercellular bridges (Fig. 379). If these thickened striae consisted indeed of "bacillary modified foci on adjacent fibrils" as Jordan and Banks (1917) describe them, transverse sections should show them as homogeneous. Our transverse sections show the discs as a network of anastomosed trabeculae thicker and more stainable than the fibrillae with clear fluid in the narrow spaces of the network. Hence in addition to modification in structure and chemical composition the fibrillae have undergone a special modification of arrangement in the discs. Instead of being united into small columns they are condensed and packed into a network of ramifying and anastomosing bands. It is possible that the clear fluid in the meshes of the network corresponds to sarcoplasm or perhaps to a

different substance colored black with silver nitrate and comparable to the intercellular substance found in an epithelium. If at the level of the discs there exists no trace of intercellular cement then the whole system must be regarded as a syncytium.

There are also intracytoplasmic discs disposed in terraced arrangement at different levels, even at that of the nuclear zone. They bear no relation to the cell limits, a characteristic also found in true cardiac cells. For further discussion of these phenomena the papers of Jordan may be consulted (Jordan and Steele, 1912; Jordan and Banks, 1912).

Finally we must mention the intercalated disc (Fig. 393, 1) which subdivides one daughter-cell alone, incompletely separated from its twin.

Schockaert (1909) has described around all the Purkinje fibers of the sheep's heart a membrane stained blue with iron hematoxylin. Jordan and Banks (1917) describe a sarcolemma around the cells of the moderator band in the ox. We have already described an endomysial membrane of connective tissue-like structure, stained blue by Mallory's method and green by light green, round both types of Purkinje cell. It is also seen round the transitional cells to be described later. In transverse section this endomysium is frequently to be seen as a deeply-staining membrane distinct from the neighboring interstitial connective tissue and continuous with the cleavage septum between the daughter-cells (Fig. 385, PT). It may attain considerable thickness and undoubtedly presents the greatest similarity to the sarcolemma often much hypertrophied and obviously of connective tissue origin, in pathological hearts.

I have already mentioned the more abundant vascular interstitial connective tissue around the true cardiac cells. Since our first paper (Van der Stricht and Todd, 1919) was published this phenomenon has been investigated again in Eliot R. Clark's laboratory (Johnstone and Wakefield, 1922) by workers who did not know these observations but who confirmed them, though they drew deductions which seem unwarrantable and their evidence was not altogether satisfactory, being based only on injection of some hearts of ungulates. Many illustrations in this article (Van der Stricht and Todd, 1919) show that there is no lack of vascularity of Purkinje fibers and indeed the vascularity of the interfascicular connective tissue was long ago emphasized by Tawara (1906).

It should also be noted at this stage that the group of workers just referred to found the fibers [cells] of the atrio-ventricular node very much smaller (as stated by Tawara) and exhibiting more branching and anastomosis than in any other region of the system. They also found the fibers [cells] of trunk and of right and left branches much larger than those of the node itself though smaller than those of the terminal expansions. In thus expressing their findings these observers confirm the current views to which we find ourselves emphatically opposed. Cells of all sizes are to be

found everywhere throughout the system and statements to the contrary are simply the result of indequate observation or material. Even in the illustrations herewith presented evidence can be gleaned of the very variable size of cell in a particular location.

VI. LONGITUDINAL SEGMENTATION OR CLEAVAGE OF PURKINJE CELLS

In 1902 Heidenhain described a process of longitudinal division of cardiac fibers into "daughter fibers" through the appearance of a system of longitudinal clefts from which all trace of transverse striation is abolished and in which appears a simple sarcolemma-like membrane.

We have found the same phenomenon in Purkinje cells and have sketched slightly in references above to the occurrence of fibrillae passing through the axial part of the cell both of "embryonic" and "adult" type, especially the latter.

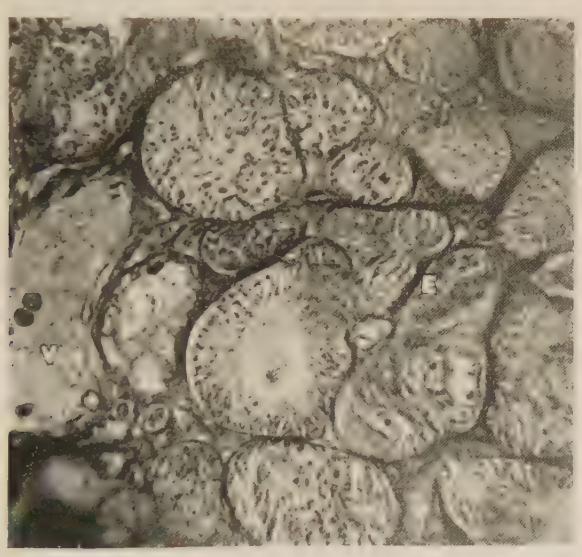
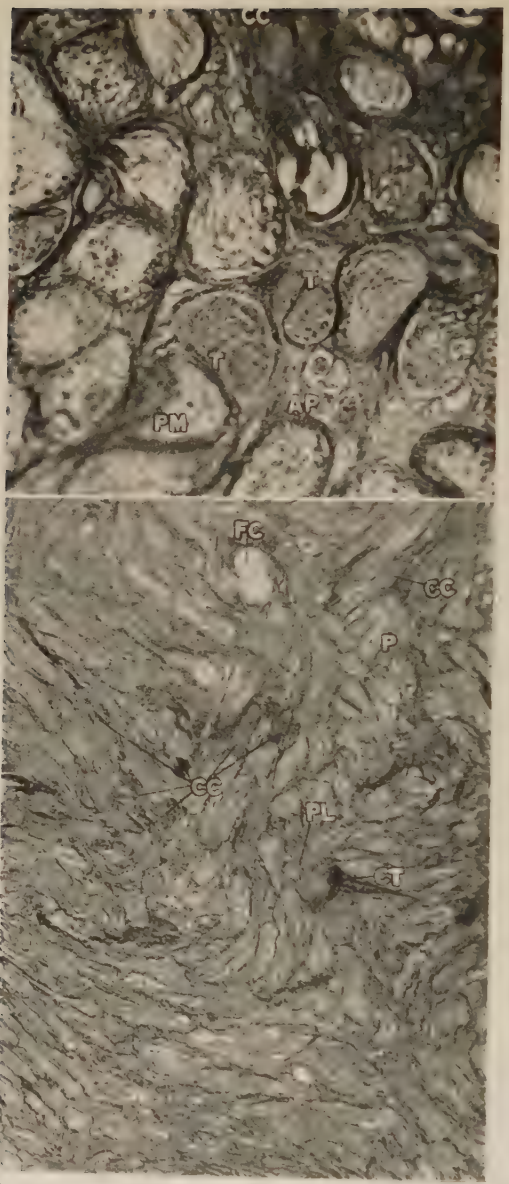


FIG. 395.—Purkinje cells from left posterior ventricular branch in interventricular septum. Syphilitic myocarditis. Age forty-two years. 535 diameters. Note the increased connective tissue and exuberant splitting of Purkinje cells into daughter-cells. E, endomysium; V, vein. (Hermann; Mallory.)

In transverse section one may discern three related forms of Purkinje cell:

1. Cells with a cortical fibrillar zone and a central granular area.
2. Cells in which the fibrillae are more or less uniformly distributed throughout the entire extent of cytoplasm.
3. Bipartite or tripartite cells. In these one or two fibrillar partitions subdivide the mother-cell into two or more lobes more or less symmetrical in size and structure (Figs. 384, 386, 395).



396

397

FIG. 396.—Purkinje cells from left posterior ventricular branch in interventricular septum. Syphilitic myocarditis. Age forty-two years. 535 diameters. Note increased connective tissue and exuberant splitting of Purkinje cells into daughter-cells. AP, atrophied Purkinje cells; CC, cardiac cell; PM, Purkinje cell splitting into daughter-cells; T, transitional cell. (Hermann; Mallory.)

FIG. 397.—Whorled meshwork of Purkinje cells from the atrioventricular node. Age thirty-six years. 103 diameters. CC, cardiac cells; CT, connective tissue; FC, fat cell; P, Purkinje cells; PL, Purkinje cell with plexiform fibrillae. (Hermann; safranin; light green.)

The subdivision occurs indeed in a normal heart but is much more active in the pathological organ. At times and especially in pathological states the lobes are asymmetrical, one half of the protoplasm containing many more fibrillae than the other and resembling closely the cytoplasm of a true cardiac cell (Fig. 396). There may even be differentiation of the axial cytoplasm to resemble that of a cardiac cell while the more peripheral zone retains the features typical of a Purkinje element (Fig. 386 cc).

In the second stage the endomysium surrounding the mother-cell proliferates into the intracytoplasmic fibrillar partition and subdivides the cell body into two or more daughter-cells (Fig. 385).

In pathological hearts this process is carried out in extreme and bizarre form and is followed by atrophy of the resulting daughter-cells. The twin elements may be no greater in transverse diameter than a smooth muscle cell and ultimately are resolved into small cytoplasmic masses no larger than a lymphocyte (Fig. 396): they may or may not be nucleated and possess occasional fibrillae. The longitudinal cleavage of Purkinje cells separates the nuclei so that daughter-cells are nucleated. But in pathological hearts there may also be segmentation phenomena or direct division of nuclei induced by extension of the proliferating connective tissue septum (Figs. 387 to 389).

Sometimes a capillary penetrates the Purkinje cell and helps to subdivide it (Fig. 394 cp). This is of course an item in the penetration by endomysium.

It is difficult to know whether division of the Purkinje cells should be regarded as amitotic or mitotic with a very long delay between the initial division of nuclei and much later cleavage of cells. For a full discussion of this problem reference should be made to the extended study of Van der Stricht and Todd, (1919).

VII. TRANSITIONAL TYPES OF CELL

Earlier in this article the Purkinje cells of "adult" type have been recognized as an intermediate form between the "embryonic" Purkinje cells and the true cardiac cells. It is now necessary to lay stress upon the occurrence in the normal heart of every stage intermediate between these two extreme forms. The "adult" Purkinje cell is the most frequently encountered variety but between it and the "embryonic" type on the one hand and the true cardiac cell on the other are to be found in considerable number throughout the Purkinje system every possible intermediate stage. It is only the transitional types between the "adult" Purkinje cell and the true cardiac cell that we need deal with here. Transition may occur gradually or at other times suddenly. In transitional cells (Fig. 390) one can see the fibrillae greater in number, the sarcoplasm diminished in amount and the transverse striation progressively more marked.

That adult Purkinje cells are actually transformed into true cardiac cells even in the normal heart rests on the following evidence:

1. Great numbers of cardiac cells are found scattered among the Purkinje cells (Fig. 397).

2. Great numbers of cardiac cells are seen to be produced in normal and especially in pathological hearts by the cleavage of mother Purkinje cells (Figs. 385, 386, 395, 396).

3. In plexuses or very complicated meshworks of cardiac cells there are found occasional Purkinje cells and transitional elements (Fig. 398).

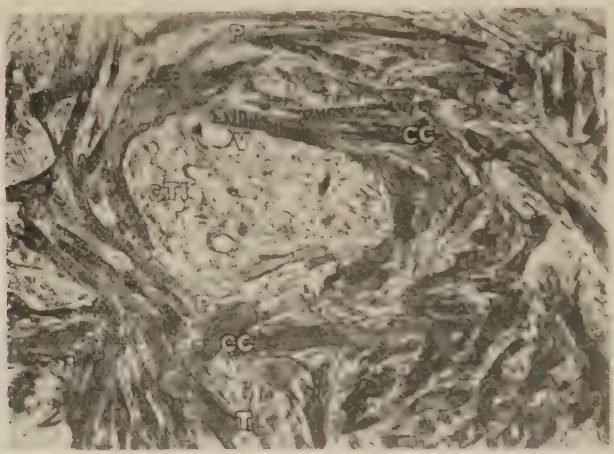


FIG. 398.—Whorled meshwork of cardiac cells from the atrioventricular node. Syphilitic myocarditis. Age forty-two years. 103 diameters. CC, cardiac cell; CTI, infiltrated connective tissue; P, Purkinje cell; T, transitional cell; V, vein. (Hermann; Mallory.)

VIII. GENERAL ARRANGEMENT OF CELLS IN THE PURKINJE SYSTEM

SUMMARY

The great mass of Purkinje tissue from the opening of the great veins to the terminal ventricular expansions consists of cells of "adult" type. These are arranged in longitudinal networks, in bundles or in flattened sheets. The first form is characteristic of the right atrium, the second of the right ventricle and the third of the left ventricle. However there is nothing invariable about this disposition; the so-called bundle of His in the right ventricle may be present only as scattered strands or perhaps a very thin flattened sheet honeycombed with large fenestrations and indistinguishable to the naked eye. The bundles or fascicles have a surrounding vascular connective tissue in which are many nerves. These fascicles are composed in the main of "adult" type cells laid end to end arranged more or less parallel with each other and with the axis of the fascicle. They are bound together by short obliquely placed branches so that the fascicles really form

networks the parallel limbs of which are much longer than the anastomosing ones. The fibers are but one cell thick. The arrangement of fibers as parallel bands, so characteristic of the pattern of cardiac cells, simply does not exist. On the other hand we find quite frequently, throughout the heart, a whorled or spiral arrangement of Purkinje cells (Fig. 397).

Purkinje cells may be of considerable, medium, or small transverse diameter: the fibrillae may be confined to a small peripheral cortical zone or distributed more or less uniformly throughout the entire cross sectional area. This distribution of fibrillae throughout the cell is undoubtedly the character which most strikingly differentiates the Purkinje cell of the atrium from that of the ventricles. On the whole atrial cells are of smaller diameter than those of the ventricles but there are many exceptions to this generalization. Differences in volume and in distribution of fibrillae are merely accessory features in a single type of cell. I cannot confirm the morphological basis of Lewis' (1921) law of cardiac muscle.

Throughout the entire Purkinje system there is distributed another type of cell, also multinuclear but shorter and polyhedral with rather smaller nuclei even less like those of the true cardiac cells. This type of cell we have called "embryonic": in it the fibrillae may be longitudinal and parallel or may be intercrossing or even plexiform. The cells may be placed end to end but most frequently they are intercalated between neighboring longitudinally arranged fibers into the substance of the cells of which their fibrillae freely pass, either through an intercalated disc or without obvious delimitation of any kind.

Mixed among the Purkinje fascicles or even forming a part of their length are true cardiac cells to which also the fibrillae of "embryonic" Purkinje cells may pass.

Plexiform or even stellate arrangements of Purkinje cells are indeed found in that part of the system named the Knoten by Tawara (1906). But we find these characteristics present throughout the entire Purkinje system from the so-called sinus node right to the ventricular expansions. I can find no special nodal tissue and do not believe that either sinus node or atrioventricular node exists as a special formation. Any specificity of these regions must be purely physiological: it has no basis in anatomical fact.

IX. EVIDENCE OF PATHOLOGY IN THE PURKINJE SYSTEM

Although this account deals with the normal cytology of the Purkinje system it is worth while briefly to indicate the evidences of a pathological nature which may be expected in routine examinations. Indeed it has not been possible to describe the regular appearances without reference to the exaggeration of normal phenomena in pathological states.

It is extremely disconcerting to find lesions of the most gross variety in the course of or even throughout the Purkinje system with no corresponding electrocardiographic features. It is equally disconcerting to be unable to discover an anatomical basis for the most flagrantly aberrant electrocardiographic records. But as I have already indicated there is a doubt regarding the complete validity of current theories relating the Purkinje system to conduction of the heart impulse the reader will not be surprised to find that no help is at present forthcoming upon this problem.

Regarding more acute forms of inflammation little remains to be said. Fatty degeneration can be demonstrated after fixing with osmic reagents. Such fatty granules occur both in the central sarcoplasmic area and in the peripheral fibrillar zone. They are present also in the normal heart however as Bullard (1916) has shown. The invasion of leucocytes usually stops at the epimysium, perimysium, and endomysium, the Purkinje cells being very resistant to invasion. It would seem that the Purkinje element is endowed with phagocytic properties for in some instances masses of leucocytes can be observed undergoing disintegration and karyolysis in the midst of a Purkinje cell. Lymphocytes with a small nucleus rich in chromatin, leucoblasts bulkier with a simple nucleus surrounded by a more abundant protoplasm, and polymorphocytes with a lobed nucleus can all be found in the connective tissue in acute inflammation, and even red cells extravasated in large numbers. "Mast-cells" are as frequent in normal as in pathological tissue.

It has already been stated that the normal processes of cleavage and of transformation into cardiac cells are greatly exaggerated in pathological states. Accompanying these phenomena is a marked increase of connective tissue in the Purkinje system amounting in places, perhaps widely distributed, to a veritable fibrosis with reduction or even total loss of Purkinje elements. These features are the most pronounced of the pathological lesions which I have seen. Tumor growth sprayed over the system need not detain us.

X. BIBLIOGRAPHY*

Bullard, H. H. 1916. On the occurrence and physiological significance of fat in the muscle fibers of the normal myocardium and atrio-ventricular system. *Am. J. Anat.*, **19**, 1.

Cady, L. D. 1921. A microscopical study of the sino-ventricular bundle of the rabbit's heart; with reference to the data relative to its functional interpretation, especially in terms of a source of replacement of degenerated myocardium. *Anat. Rec.*, **21**, 377.

* This list is not exhaustive. It is impossible to encompass the entire literature but the references given will guide the reader to the main sources of information. Many articles, especially the more recent ones, are of inferior quality and misleading character. They are purposely omitted.

De Witt, L. 1909. Observations on the sino-ventricular connecting system of the mammalian heart. *Anat. Rec.*, **3**, 50.

Eyster, J. A. E. and **Meek, W. J.** 1921. The origin and conduction of the heart beat. *Physiol. Reviews*, **1**, 1.

Fahr. 1907. Ueber die muskuläre Verbindung zwischen Vorhof und Ventrikel (das His'sche Bündel) in normalen Herzen und beim Adams-Stokes'schen Symptomkomplex. *Virchow's Archiv*, **188**, (F. 18, Bd. 8), 562.

——— 1908. Zur frage der atrioventrikulären Muskelverbindung im Herzen. *Verhandl. d. deutsch. path. Gesellsch*, **12**, 153.

Heidenhain, M. 1902. Ueber die Struktur des menschlichen Herzmuskels. *Anat. Anz.*, **20**, 33.

His, W. Jr. 1893. Die Tätigkeit des embryonalen Herzens und deren Bedeutung für die Lehre von der Herzbewegung beim Erwachsenen. *Arb. a.d. med. Klin. z. Leipz.*, **14**.

Hoyer, H. 1899. O budowie i podziale komórek mieśnia sercowego (Ueber die Struktur und Kerntheilung der Herzmuskelzellen) (Vorl. Mitt.). *Bull. de l'Acad. des Sc., Cracovie*, 487.

——— 1901. Ueber die Kontinuität der kontraktilen Fibrillen in den Herzmuskelzellen. *Ibid*, 203.

Ingalls, N. W. 190-. Beschreibung eines menschlichen Embryos von 4.9 mm. *Arch. f. mikr. Anat. u. Entw.*, **70**, 506.

——— 1920. A human embryo at the beginning of segmentation with special reference to the vascular system. *Contrib. to Embryol. (Carnegie Inst. Wash., Pub. No. 274)*, **52**, 71.

Johnstone, P. N. 1923. Studies of the atrio-ventricular bundle with polarized light. *Anat. Rec.*, **26**, 145.

Johnstone, P. N. and **Wakefield, F. H.** 1922. On the character of the Purkinje fibers in various regions of the atrio-ventricular bundle. *Anat. Rec.*, **24**, 223.

Johnstone P. N., Wakefield, F. H. and **Currey, H. M.** 1922. On the comparative vascularity of heart muscle and of the Purkinje fibers. *Anat. Rec.*, **24**, 55.

Jordan, H. E. and **Banks, J. B.** 191-. A study of the intercalated discs of the heart of the beef. *Am. J. Anat.*, **22**, 285.

Jordan, H. E. and **Steele, K. B.** 1912. A comparative microscopic study of the intercalated discs of vertebrate heart muscle. *Am. J. Anat.*, **13**, 151.

Keith, A. and **Flack, M.** 190-. The form and nature of the muscular connections between the primary divisions of the vertebrate heart. *J. Anat. & Physiol.*, **41**, 172.

Kent, A. F. S. 1893. Researches on the structure and function of the mammalian heart. *J. Physiol.*, **14**, 233. (Actually Nov. 12, 1892.)

——— 1914*a*. The right lateral auriculo-ventricular junction of the heart. *Ibid.*, 48, xxii.

——— 1914*b*. A conducting path between the right auricle and the external wall of the right ventricle in the heart of the mammal. *Ibid*, 48, lvii.

——— 1914*c*. Illustrations of the right lateral auriculo-ventricular junction in the heart. *Ibid*, 48, lxiii.

Koch, W. 1922. *Der funktionelle Bau des menschlichen Herzens.* Berl: Urban and Schwarzenberg.

Lewis, T. 1921. The law of cardiac muscle with special reference to the mammalian heart. *Quart. J. Med.*, **14**, 339.

Lhamon, R. M. 1912. The sheath of the sino-ventricular bundle. *Am. J. Anat.*, **13**, 55.

Mackenzie, I. 1913. The excitatory and connecting muscular system of the heart. *XVIIth Internat. Congr. of Med.*, Sect. I, Pt., 1,121.

Mall, F. P. 1902. On the development of the connective tissues from the connective tissue syncytium. *Am. J. Anat.*, **1**, 338.

——— 1912. On the development of the human heart. *Am. J. Anat.*, **13**, 249.

Marceau, F. 1902. Recherches sur l'histologie et le développement comparés des fibres de Purkinje et des fibres cardiaques. *Thèse de Nancy.*

Meiklejohn, J. 1914. On the innervation of the nodal tissue of the mammalian heart *J. Anat. & Physiol.*, **48**, 1.

Mönckeberg, J. G. 1908. *Untersuchungen über das Atrioventrikularbundel in menschlichen Herzen.* 1 Jena.

Obermeier, O. H. F. 1866. *De filamentis Purkinianis. Questio academica praemio coronatu.* Dissert. Inaug. Berl: G. Schade.

——— 1867. Ueber Structur und Textur der Purkinje'schen Fäden. *Arch. f. Anat. Physiol. u. Wissensch. Med.*, Leipz., *245*, 358.

Oppenheimer, B. S. 1912. A routine method of opening the heart with conservation of the bundle of His and the sino-auricular node. *J. Am. M. Ass.*, **59**, 937.

Rénon, L. 1914. Note à propos de la discussion sur la faisceau de His. Richesse en fibrilles élastiques. *XVIIth Internat. Congr. Med.*, Sect. I, Pt. 2, 22.

Retzer, R. 1920. The sino-ventricular bundle: a functional interpretation of morphological findings. *Contrib. to Embryol. (Carnegie Inst. Wash., Pub. No. 2-2)*, **32**, 143.

Sabin. Cited by Mall, 1902.

Schockaert, A. 1909. Nouvelles recherches comparatives sur la texture et le développement du myocarde chez les vertébrés. *Arch. de Biol.*, **24**, 277.

Tawara, S. 1906. *Das Reizleitungssystem des Säugetierherzen.* Jena:

——— 1906. Ueber die sogenannten abnormen Sehnenfäden des Herzens: ein Beitrag zur Pathologie des Reizleitungssystems des Herzens. *Beitr. z. path. Anat. u. z. Allg. Path.*, **39**, 563.

Tufts, J. N. 1921. Some observations upon structure of the Purkinje fibers. *Anat. Rec.*, **22**, 363.

Van der Stricht, O. and Todd, T. W. 1919. The structure of normal fibers of Purkinje in the adult human heart and their pathological alteration in syphilitic myocarditis. *Johns Hopkins Hosp. Reports*, **19**, 1.

Wenckebach, K. F. 1906. Beitrage zur Kenntnis der menschlichen Herztatigkeit. *Arch. f. (Anat. u.) Physiol.*, Jahrg. 1906, 397.

Wilson, J. G. 1909. The nerves of the atrio-ventricular bundle. *Proc. Roy. Soc. Lond.*, 81, 151.

Section XXV

VISUAL CELLS AND RETINAL PIGMENT

CONTENTS

Section XXV

PAGE

I. Visual Cells 889
1. Visual rods 891
Outer segment—Inner segment—Coloring matter of rods
2. Visual cones 895
Outer segment—Inner segment—Double cones and twin cones—Coloring matter of cones.
3. Rod and cone fibrillae 897
Axial fiber—Spiral fibers—Coarse peripheral fiber—Fine peripheral fibers
4. Development 899
II. Pigmented Epithelium 899
1. Component zones 900
2. Cell inclusions 901
Fuscin—Guanin—Lipochrin—Myeloidin.
3. Origin of pigment 903
III. Functional Activities of Retinal Elements 904
1. Untenable theories of vision 904
2. Photochemical theory of vision 905
3. Functional interpretation of visual apparatus 907
4. Duplicity theory and correlated retinal adaptations 908
5. Color vision 911
6. Miscellaneous activities 912
7. Adaptations to life habits 913
IV. Bibliography 914

Section XXV

VISUAL CELLS AND RETINAL PIGMENT*

LESLIE BRAINERD AREY

INTEREST in the structure and function of the retina centers chiefly on the visual cells and pigmented epithelium as the elements concerned with photic reception and the transformation of light rays into the nerve impulse. Since this interest also reflects correctly the relative importance of these structures, the following account is restricted to a statement of some of the more outstanding features of their anatomy and physiology. The technical difficulties inherent in the analysis of visual cell structure have resulted in a voluminous literature of uneven value. Unfortunately, spatial limitations will make it necessary to set down rather dogmatically many conclusions without adequate discussion of the reasons for their adoption.

The best presentation of the status of retinal structure prior to 1900 is found in an admirable contribution by Greeff (1900). The physiology of the visual cells and retinal pigment is likewise extensively reviewed by Garten (1907) and the photomechanical aspects more concisely by Arey (1915*a*). Although important facts have since been added, many crucial points await solution and our knowledge of the apparatus as a working mechanism is still incomplete.

I. THE VISUAL CELLS

The rod and cone visual cells are comparable in general organization but each has specific characteristics. The external limiting membrane is a fenestrated partition through whose pores the visual elements extend. It lies about midway of the cells and so divides them into highly differentiated visual rods and cones peripherally, and into nuclear and filamentous regions centrally. Each cell consists of the following parts (Figs. 414 and 428): (1) outer segment; (2) inner segment; (3) nucleus; (4) fiber, and (5) termination.

The terminations stand in relation to neurons of the second order in the outer reticular layer. In bony fishes, nocturnal birds and mammals the rod cells terminate in an end knob, whereas in amphibia, reptiles and diurnal birds, the swollen end gives off short processes (cf. Cajal, 1894; 1911); the ending of the cone cell is the so-called cone foot which is conical in shape and divides into rootlets. The slender visual cell fiber is much finer in the rod—than in the cone cell and bears varicosities, while that of the cone cell is smooth. The cone nucleus of mammals and most fishes lies closer to the external limiting membrane and is considerably larger than the rod nucleus;

*Figures in this section will be found on plates following bibliography.

on the contrary, in amphibia, reptiles and birds the distinguishing characteristics between rod and cone nuclei are small. The mammalian rod nucelus has long been described as composed of alternate horizontal plates of chromatin. This is, at best, a gross over-statement; it is but superficially suggested in some forms and finds little or no representation in others, including man (Flemming, 1898; Schaper, 1899; Greeff, 1900). The rods and cones comprise the remainder of the visual cells; each is divisible into inner and outer segments, and these will be described fully in subsequent paragraphs.

The rod and cone elements are regarded as specialized neuroepithelial cells and not as nerve cells. To this conclusion attest a number of characteristics such as the epithelioid appearance of their outer parts and their position as boundary cells in the cavity of the primitive optic vesicle (cf. the embryonic ependyma). Development also supports the conclusion that they are specialized cells, distinct from nerve and neuroglia (Cajal, 1898). In this connection should be mentioned Krause's belief that the rods and cones are modified ependyma and the interpretation of Barker that they constitute a sensory epithelium to which the bipolar cells stand in the relation of spinal ganglia.

From time to time various authorities have referred to the outer segment of the rod and cone, or even to the entire element, as a cuticular or secreted substance, and this conception has recently been revived by Levi (1914). Their delicate composition, extreme sensitiveness and specialized structure, including problematical fibrillae (p. 89–), apparently negative this view. An interpretation of the rods and cones as robust cilia, homologous with those of the central canal of the embryonic neural tube, likewise does not appeal.

It has often been discussed whether the rods and cones are distinct elements (Steinlin, 1868) and whether intermediate forms occur (Osawa, 1898; Fritsch, 1904, 1911*a*, *b*). To be sure, various characteristics of the one can be found in certain representatives of the other (e.g., the rods of *Triton* possess conical outer segments; cones of birds have inner segments scarcely thicker than those of rods; in man the cones in the fovea resemble the rods at the retinal periphery; the rod cells of some animals connect with the bipolar cells like cones), yet there are always sufficient critical structural characteristics to allow the two to be differentiated (Laurens and Detwiler, 1921; Pütter, 1912). From a histogenetic standpoint, the cones have commonly been considered as specialized rods whose development has extended farther (Parsons, 1915; Kerr, 1919). On the contrary, Bernard (1903) and Cameron (1905; 1911) conclude that the cones represent early stages in the formation of rods. Another and sounder view (Levi, 1901, 1914; Leboucq, 1909; Magitot, 1910; Seefelder, 1910; Cajal, 1911; Detwiler and Laurens, 1921) is that both the rod and cone cells develop

simultaneously and differentiate divergently from a relatively indifferent progenitor. The course of development of the visual cells and the intricate and contested details of differentiation may be found in the works of these authors.

Champy (1914) cultivated the retinas of the rabbit and turtle by the tissue culture method. Although the sensory and nervous elements degenerated rather promptly, the pigmented epithelium, Müller's fibers and neuroglia continued to live and grow. The pigmented epithelium of the chick has also been successfully cultured by Luna (1917) and by Smith (1920).

The relative distribution of visual cells in most fishes, amphibia and mammals is such that there are more rods than cones, but in birds the reverse occurs. The retina of most diurnal reptiles is composed exclusively of cones. On the contrary there are nocturnal vertebrates which lack cones (e.g., the ray and shark, certain geckos, hedgehog, bat, mole and a monkey); yet there are other nocturnal animals, as the owl, mouse, dormouse and rat, that have a few rudimentary cones.

The human macula and fovea possess nothing but cones, those of the fovea alone totaling 4000. In the remainder of the retina the mosaic of rods and cones is very regular; near the macula the cones are separated from each other by a single circle of rods; a short distance from the macula each cone is surrounded by series of rods three to four rows deep, and this pattern continues even close to the ora serrata. The total number of rods in the human retina totals 130 million, the cones 7 million (Krause).

1. *Visual rods:*

The size, shape and proportions vary greatly in the various vertebrate groups (Figs. 399-411), but the structural plan is essentially identical. Some are short cylinders (urodeles), others long cylinders (anura; birds; man) and still others even filamentous (fishes; rat). The outer and inner segments may be about equal in diameter (Fig. 401) or either one may be thicker than the other (Figs. 403 and 408). Similar combinations exist for the relative lengths of these parts (Figs. 409, 410 and 411). Each rod has two portions quite unlike structurally and chemically (Fig. 399): (1) the strongly refractive outer segment which blackens with osmic acid and tends to stain preferentially with acid dyes; (2) the finely granular or almost homogeneous inner segment which takes basic dyes. The chemical differences between the outer and inner segments of the rods, as well as the constitution of the cones and pigmented epithelium, have been studied by Lo Cascio (1923); the staining characteristics and the variations after adaptation in darkness and light are recorded by Fujita (1921*a*, *b*) and Majima (1925). The outer segment is doubly refractive to polarized light, the inner segment singly (Howard, 1908). Between the two divisions of the rod in many animals there is a narrow, light zone which may be termed the intermediate plate (Fig. 399) (Schultze, 1867; Greeff, 1900); this serves as a barrier,

except at the periphery of the elements, where a continuity between the two members is maintained (Howard, 1908). A discussion of the interesting fibrillae of rods and cones will be given later (p. 897).

Human rods have a length of 60μ in the fundus region; toward the periphery of the retina they shorten somewhat to 50μ and measure but 40μ at the ora serrata. The diameter is about 2μ.

a. Outer Segment. The form in man is purely cylindrical (Fig. 411). The base is squarely at right angles to the long axis; the outer end arches slightly but in some animals (frog, hog) it is more convexly domed (Figs. 402 and 410). The surface of the cylinder is scored by delicate, parallel furrows coursing lengthwise. In amphibia such furrows are plainer and may be seen in unfixed preparations; in these animals they slant and so appear slightly spiral (Fig. 402). Transverse sections, as well as plates separated from fresh or osmic preparations by teasing, show the rim to be finely scalloped with elevations alternating with depressions (Fig. 414). The grooves are commonly interpreted as impressions caused by the processes of the pigmented epithelium, but this may properly be questioned. The ridges have been described as bearing longitudinal fibrillae (p. 898, Figs. 401 and 412).

Each outer segment consists of a containing sheath and of an interior of superimposed plates bound together by thin layers of cementing substance (Figs. 405 and 414). The sheath is of a cuticular nature. It is demonstrably continuous with the delicate sheath of the inner member, which in turn apparently unites with the external limiting membrane (Kuhnt, 18--); the sheath, however, does not enclose the outer end of the rod. The interior of the outer segment appears in the fresh condition as entirely homogeneous and strongly refractive; nevertheless, even in the most favorable examining medium, such as aqueous humor, there soon enter characteristic and highly instructive degenerative phenomena. The first noticeable change is an appearance of cross banding of the outer segment, accompanied by swelling; presently a number of discrete plates, equal in thickness, can be detected and the outer member bends (Fig. 413) and finally liberates the plates into the examining fluid (Fig. 414). The thickness of the plates in man is about 0.5μ and there is little variation from this figure in other animals. Such plate layering has been interpreted by some as a close spiral structure (Krause, 1893; Ritter, 1891*b*; Moroff, 1922), but this seems unlikely.

Another post-mortem change, which is induced equally by imperfect fixation, heat or subjection to water, involves a bulbous swelling and fragmentation of the outer segment, resulting in degenerative droplet formation (Fig. 415). This reaction, as well as the physical and microchemical characteristics of the droplets formed, closely resembles that of myelin of nerve fibers. The two are not identical, as the color reactions to osmic acid show (Kühne, 1879), yet this myeloid substance of the outer segment and

the myeloid bodies of the pigmented epithelium (p. 903) seem to stand closest of all the body constituents to the myelin of the nervous system. The intensity of the reaction to osmic acid varies greatly in the rods of different animal groups and may vary even among the rods of the same individual (e.g., frog); differences in coloration are observed also with such stains as the Ehrlich-Biondi mixture (Arey, 1916c).

b. Inner Segment. In man the inner segment is three-fourths as long as the outer segment but is somewhat thicker and spindle-shaped (Fig. 411). It is composed of a remarkably transparent cytoplasm which soon becomes cloudy and granular. Basally there are delicate longitudinal striations; these are due to fine basket fibrils which are terminations of Müller's fibers extending beyond the external limiting membrane and apposed closely to the rods and cones. Such fibrils are in part separable and obviously do not belong properly to the inner segment.

a. Lentiform Body. In the inner segment, near its junction with the outer, is an apparatus which is usually called the ellipsoid, but since that shape is uncommon, the term lentiform body is preferable. In fishes and anuran amphibia it is a single plano-convex element, truncated on its distal surface (Figs. 399 and 402). In reptiles (Figs. 405 and 406) and birds (Fig. 408) it is more complex, and especially in salamanders there is a proximal biconvex "paraboloid" in addition to the plano-concave "ellipsoid" (Fig. 401). Mammals possess a modification known as the "fiber apparatus;" it occupies the outer third of the inner segment and consists of many fine refractive chromophilic fibers, which, near the surface, course regularly parallel to the long axis but which in the interior decussate frequently (Fig. 411).

Lentiform bodies may well play a dioptric rôle (p. 907). Many of their properties suggest this, and the ellipsoid-paraboloid combination is most interesting from such a viewpoint. Its components have geometrical forms like those of the component lenses in an achromatic objective, and both are more refractive than the surrounding medium; the paraboloid has little affinity for stains, is comparatively stable and persistently clear after death and has the lower index of refraction; on the contrary, the ellipsoid is strongly chromophilic, shows marked instability with rapid clouding at death and possesses a higher index of refraction (Howard, 1908).

b. Myoid. The inner segment of the rods of many animals shows a remarkable power of elongation and contraction under different physical conditions (p. 907). This is not a property of the lentiform body but of the remaining protoplasm which has been given the appropriate name "myoid" (Figs. 399 and 403). That the mobility may result in a 90 per cent change in length (Arey, 1915*a*; 1916*a*) is all the more remarkable in the absence of apparent specialization for this purpose (Figs. 448 and 449).

c. Coloring Matter of Rods. Visual purple occurs in the rods of vertebrates from *Petromyzon* to man; it is lacking in some bats and some diurnal birds but is abundant in most nocturnal animals. It is absent from all invertebrates. Visual purple has long been held to occur only in the outer segments of rods; in man the rods near the ora serrata lack it and visual purple is quite naturally absent in the rod-free fovea (but cf. p. 896 and the observations of Hecht and Williams, 1922). In animals possessing a retinal tapetum (e.g., the fish, *Abramis*) visual purple and its fading in light can be observed in the living eye (Abelsdorff, 1898).

Some anuran rods are green in color. The outer segment is short, the inner segment long, so these are easy to identify (Fig. 403). The coloring matter is said to be a modification of visual purple, called visual green, which bleaches more slowly and leaves the outer segment less transparent than in red rods. On the contrary, it has never been extracted and possibly does not exist as a distinct substance; the green color may be purely a contrast phenomenon. Nevertheless, the green rods are chemically unlike the red, as is evidenced by the differential staining of their outer segments.

Kolmer (1909; 1914), with a modification of Held's fixative, demonstrated droplets which usually lay on the surface of rods (Fig. 406), and rarely on cones, of fishes, amphibia, birds and mammals (including monkeys and man). These, he asserted, are not artefacts but true secretion products of the pigmented cells, derived from the myeloid bodies (p. 903) which agree in shape and color but stain more feebly. The droplets vary in size and form in different animals but there is a direct correlation with the size of the visual elements concerned. They were not found in the lizard and diminished to the vanishing point in the center of the mammalian macula; this parallels the occurrence of visual purple. Droplets are absent in retinas adapted to light but are abundant in those that have remained in the dark. The use of pilocarpin hastens their reappearance in retinas brought from the light into the dark, and actual extrusion from the pigmented cell was then claimed to be observed. All these relations recall the distribution and properties of visual purple of which Kolmer surmises these bodies may be precursors. Detwiler (1923) has confirmed the occurrence of such droplets in the cone-free retina of the gecko, and in the field mouse and frog, and failed to find them in the rodless retina of an Asiatic lizard. He considers that they stand in genetic relation to granular material in the rod's outer segment (Figs. 405 and 406), rather than to the pigmented cells, and suggests a probable relation with visual purple and the visual function of the photoreceptors. In a recent note Kolmer (1925) not only abandons his former view and assents to Detwiler's interpretation of a droplet origin from the rod's outer segment, but also holds it a matter of experimental proof that the acetic acid of the fixatives used is responsible for their ready

loss from rods and less easy extraction from cones. It remains to be proved that all the known data are amenable to such an explanation.

2. *Visual cones:*

The name describes well the shape of these conical elements in man and many other animals, but in some, and especially those whose cones exhibit considerable mobility, it is not so appropriate. Physically and chemically, the homologous parts of rods and cones agree, but in gross form there is marked dissimilarity (Figs. 416 to 430).

There is a great variation in the shape of human cones correlated with their position in the retina (Figs. 42– to 430). The foveal cones are long, slender and rod-like, measuring 85μ in length and 2.5μ in diameter; at the periphery of the macula they have shortened and broadened considerably and in the fundus they are 32μ long by 7μ broad; near the ora they have shortened still more until the smallest are 22μ by 7.5μ.

a. Outer Segment. The shape is typically conical, although the pointed tip is commonly not preserved, due to its extreme fragility (Figs. 416, 419 and 420). In certain forms, however, the outer segment is much elongated (Figs. 422 and 425) and in the human retina there are notable regional variations (Figs. 42– to 430). The sheath, unlike that of rods, continues over the outer member and encloses the tip. The interior content appears homogeneous when in the fresh condition; it is strongly refractive, does not stain with basic dyes and degenerates more readily than the corresponding material of the rod's outer segment. Platelet degeneration occurs frequently, yet the plates remain better in place, perhaps because of the complete sheath (Fig. 431). Droplet degeneration occurs under the same conditions as in rods but even more easily. A channeling of the outer surface as in rods occurs, at least in some animals, but with grooves nearer together; this would speak against the view that both are caused by the processes of pigment cells.

b. Inner Segment. This part of the cone is usually much bulkier than the outer segment which merely forms a tip or cap to it. Especially striking is the difference in length between these segments in the elongated phase of cones that have a high degree of extensibility (Figs. 449 and 450). The inner segment consists of pale, delicate protoplasm which soon becomes finely granular after death; it stains strongly with basic dyes. The inner segment is enclosed by a sheath, although its existence was long doubted. Basket fibers extend upward from the external limiting membrane, as in rods, but they are more abundant and easier to demonstrate.

Luna (1911*b*), Brammertz (1914) and Majima (1925) have advanced microchemical proof of the presence of glycogen in the rod and cone layer of representative animals. It occurs mostly in the inner segment, and, at least in the frog, chiefly in the ellipsoid. Its abundance is not correlated

with a previous adaptation to light or darkness or to fasting of a month's duration. The oil globules and diffuse pigment that characterize certain cones are described in a subsequent paragraph.

Lentiform Body. Peripherally, next the outer segment, the cone contains a prominent lentiform body or ellipsoid which in mammals comprises two-thirds of the inner segment (Figs. 416 to 430); only in some reptiles is it missing. Certain amphibia and reptiles possess an ellipsoid-paraboloid combination (Figs. 419 and 423). In man it is composed of a felt-work of extraordinarily fine and delicate fibers that intertwine in an oblique spiral (Fig. 428).

b. Myoid. The cones possess a myoid similar to the rods but in most animals the myoid of the cones shows even more mobility (Figs. 449 and 450). The maximum ratio of an extended to a retracted myoid is as 10:1 (Arey, 1915*a*; 1916*a*).

c. Double Cones and Twin Cones. These occur in the retinas of all vertebrates except mammals. They consist of two cones whose inner segments are united. For the most part they are the so-called double cones; in this association one member is larger and so is designated the chief (or far) cone, whereas its smaller mate is the accessory (or near) cone (Figs. 421 and 424). Each element shows characteristic structural and microchemical (i.e., staining) properties; the chief cone is of the ordinary sort, the accessory is considerably modified. The nuclei and fibers, like the outer segments, are unfused. Other cones, of equal size, unite broadly throughout their inner segments and are true twin cones (Fig. 418). Those of fishes occur only in forms with well-developed eyes and never in nocturnal fishes with little eyes; this leads Wunder (1925) to believe that they make for better vision.

Dobrowolsky (1871) interpreted double cones as actual stages in division, but this view is untenable. Although Levi's (1901) opinion that they are incomplete division forms from a single embryonic cell seems plausible, the suggestion of Detwiler and Laurens (1921) that double cones result from the fusion of single pre-existing elements perhaps explains better all conditions of double and twin cones; the latter view is consistent with the recorded total absence of mitotic figures at the stage when double cones first appear.

d. Coloring Matter of Cones. Most cones are colorless, and it has long been claimed that none contain visual purple; however, Weigert (1921) and especially Hecht and Williams (1922) believe it may be present in great dilution because there is evidence of an almost identical photosensitive substance in the cones. On the contrary, the cones of birds and certain reptiles and amphibia contain a strikingly colored oil globule and there may be diffuse red, yellow or yellow-green pigment throughout the inner segments of some birds and reptiles. The oil globule, colored or colorless, occupies the entire breadth of the inner segment near its junction with

the outer segment (Figs. 420 to 426); all colors seem to be represented, but those toward the blue-violet end of the spectrum are rare. The colored globules are most striking in birds, where Waelchli (1883) distinguished some eight varieties. Certain orderly arrangements in regional distribution may be discerned from center to periphery of the retina, thereby constituting such areas as the "red field" and "yellow field;" even more impressive is the vertical stratification with definite globules at different levels. The colored globules consist of a colorless base, which represents the sole constituent of the uncolored globules, and of a coloring material impregnating it. The strongly refractive globules exhibit a fat-like nature.

3. *Rod and cone fibrillae:*

Among the most interesting features of visual cell structure are the fibers that various investigators have described. What they signify—whether real or artefact and whether inert supporting or neuroid in nature—is not clearly established. The fibers fall into four categories:

a. Axial Fiber. This is commonly known as "Ritter's fiber" and has been described and denied by numerous investigators as a real constituent of the rod outer segment. Recent affirmations are by Lebouceq (1909) and Fritsch (1911*a*, *b*) who record the presence of an axial fiber in the outer segments of both rods and cones; this strand is traced by Lebouceq to an ending in one of a pair of diplosome granules at the ellipsoid. In the inner segments of both rods and cones axial filaments also have been described. Ritter (1859) viewed the rod as a myelinated nerve fiber whose axis cylinder is the strand that bears his name, and this interpretation, supported by many writers and investigators even to recent times (Johnson, 1896), had a long vogue and considerable influence on the concept of nerve endings. Greeff (1900) maintains that since a central strand can neither be isolated nor observed in the separated plates (p. 895) it does not exist in life, and that the axis that sometimes is seen in stained preparations is nothing else than the result of unequal fixation on an outer segment which has a softer core and firmer periphery (Fig. 414). Nevertheless, that there may be a normal regional differentiation of the outer segment is supported by Howard's (1908) demonstration, with toluidine blue, of an axial core in fresh rods. Probably none of these central elements has any particular functional significance.

b. Spiral Fibers. Coarse spiral filaments, single, double or even multiple, have been described by Ritter (1891*a*, *b*), Krause (1892), Hesse (1904), Howard (1908), Moroff (1922), Cattaneo (1922) and others. Hesse, in particular, reported the presence of a double system of fibers in both rods and cones of fishes, amphibia and reptiles, which extends from one end of the cell to the other (Fig. 420). That the plate structure of the outer segment is merely an appearance caused by such close spirals (Krause) is

untenable. Some of the figures published are unconvincing, yet actual spiral systems appear to be reasonably proved.

c. Coarse Peripheral Fiber. In the outer segment, Fürst (1904) demonstrated a peripheral fiber connected to a diplosome. This was confirmed independently by Kolmer (1904; 1914) and Held (1904) who demonstrated a stout fiber in the outer segments of both rods and cones, its connection with one element of a diplosome and a finer fiber extending downward from the other diplosome through the inner segment (Figs. 411 and 428). Retzius (1905) gave complete descriptions of this apparatus in the selachian rod. Such a system has been traced throughout the extent of the rod, has been observed in both rods and cones, is described for the embryo, newborn and adult, and in the shark, frog, rabbit and man.

d. Fine Peripheral Fibers. These are slender filaments observed by Schneider (1902), Hesse (1904) and particularly by Howard (1908) who studied them extensively. The last-named worker demonstrated elements of a fibrillar nature extending throughout the entire length of the visual cells (Figs. 401, 412 and 419). In the outer segments of the rods of *Necturus* they number between twenty and thirty and project slightly above the surface so as to produce a characteristic longitudinal ribbing. The present writer has viewed these preparations of Howard and has duplicated certain of them. Although it is theoretically possible that they do not exist as fibers in the living cell, the contrary is probably true. Howard supplemented his histological studies with interesting tests with polarized light which proved that the outer segments of the rods of many animals show phenomena indicating that they are of a fibrous nature with their positive optical direction parallel to the long axis.

It appears from the foregoing statements that several distinct types of fiber occur in visual cells: (1) spiral; (2) coarse peripheral; (3) fine peripheral. Howard has advanced the suggestion that since the single peripheral fiber has been demonstrated chiefly with silver impregnation, the one, or in some cases two, fibers thus impregnated might be due to the notably selective action of this method on individual elements of the multiple series of peripheral fibrils which he favors. Against this explanation is the fact that Fürst differentiated the single, coarse peripheral fiber with hematoxylin; furthermore, its greater size and diplosome connection which is not demonstrated in the multiple fibers of Howard compel us to assume for the present that the two types are distinct. Hesse advocates the simultaneous occurrence of fine longitudinal fibrils and coarser spiral ones, so there may truly exist several fiber types.

Any functional interpretation of the fibrils is clearly without definite supporting proof. That the finer ones are neurofibrils is most tempting, and their similarity to those of the invertebrate rhabdome encourages this

view. On the contrary, Hesse without adequate reason considers the delicate longitudinal fibers as mechanically supporting and the spiral fibers as conducting elements. Retzius records the observation that in torn preparations the single large fiber is tougher—a sort of supporting fiber. Whether any of the fibers are neuroid, and if so which, must await more critical evidence.

4. *Development:*

The method of differentiation of mammalian visual cells as advocated by Lebouceq (1909) has been confirmed and extended to man by Magitot (1910) and also by Seefelder (1914). In the early stages mitochondria are prominent and Lebouceq (1909), Magitot (1910), Leplat (1913) and Detwiler and Laurens (1921) agree that the granular material of the plates in the outer segment is of mitochondrial origin (Figs. 405 and 431). Mawas (1910) believes he has identified diffuse mitochondrial substance in the outer segments of adults as well as filaments of the same material in the inner segments. On the contrary, Levi (1914) denies its presence except as a concentration in the lentiform body.

II. THE PIGMENTED EPITHELIUM

This stratum consists of a single-layered sheet of regularly prismatic cells, mostly hexagonal (Fig. 433); it rests on the vitreous lamina of the chorioid which thus serves as a *membrana propria* for it. The bases of the component cells are enclosed by a cuticular cap which continues as intercellular cement between the adjacent cells to the level where their dense pigment content begins (Kuhnt, 1877) (Fig. 434). Fusion with the cement of neighboring cells amalgamates this intercellular product into a continuous honeycomb (Angelucci, 1878; Kühne, 1879). Verhoeff (1903) has called attention to a delicate but distinct line that may be seen in vertical sections of the retina passing across the pigment cells at the bases of the processes (Fig. 435). The line may appear intermittent and in reality is an edge view of a series of hexagonal loops which encircle the cells. Although Verhoeff insists they have nothing to do with the intercellular cement, they are obviously comparable to terminal bar phenomena and mark the distal extent of the cementing material. The writer has frequently observed the same structures in fishes, where Howard (1908) also records their presence. In this connection it may be noted that the studies of Lebouceq (1909) and Van der Stricht (1922) demonstrate that the external limiting membrane is formed by terminal bars of cement and not by Müller's fibers themselves.

The diameter of the human pigmented epithelial cell varies between 12 and 18μ, according to its location. In the region of the macula these cells are tall and slender and especially deeply pigmented; also the union of their processes with the visual elements is more intimate here than else-

where. Toward the retinal periphery the pigmented cells become broader and lower, and the regularity of their mosaic pattern is lost. In the fovea there are about nine tightly-packed cones to a pigment cell; in the remainder of the retina the ratio of visual cells is greater. The normal death and replacement of the pigmented epithelial cells have not been established (Krückmann, 1899); although Kotschetow (1908) describes multipolar amitoses and division with nuclear fragmentation, it is, not clear in the only account available to me whether fetal or mature specimens were studied.

1. *Component zones:*

Each cell shows three regions which are convenient to recognize, especially for descriptive purposes (Figs. 434 and 449): (1) nearest the chorioid, the *base*, unpigmented and containing the nucleus; (2) the pigmented *body*, an intermediate zone; (3) the *processes*, extending to a variable distance toward the external limiting membrane.

The base contains a colorless, pigment-free cytoplasm which at times appears striate; it is very delicate and soluble. The ellipsoidal nucleus occupies this part of the cell, its long axis lying parallel with the chorioid; there are one to two nucleoli, and sometimes even two nuclei (Fig. 433). The body has the typical hexagonally prismatic form; it includes much of the pigment and thereby is colored deep brown. Farthest from the chorioid are the processes, usually delicate, cilia-like protoplasmic extensions which penetrate between the rods and cones.

Krückmann (1899) has elaborated and emphasized Tschermak's (1898) incidental remark that the visual elements extend into facet-like cavities of the pigment cells so that they are enveloped, honeycomb fashion, by the recessed pigment cell. In any event the processes do not unite intimately with each other or with the rods and cones, but form protoplasmic filaments which surround the latter and are said to leave longitudinal furrows on their surfaces (p. 892), although this is open to grave doubt. The number of pigment processes varies, and, according to Angelucci (1878), includes between 10 and 40 to a cell; Wunder (1925) records that fine processes occur mostly in the retinas of fishes with small, slender rods (Fig. 448), whereas those retinas with huge cones may have a single pigment cell clasping the outer segment (Fig. 447). As the result of teasing, Angelucci (1878), Kühne (1879) and Chittenden (1879) agree that the pale processes in lower vertebrates may extend nearly to the external limiting membrane, even though the pigment is found only in their proximal part; in mammals the processes are said to be short (Greeff, 1900), but this probably is not safely established.

It is puzzling to attempt to determine whether the pigment processes are protruded and withdrawn like pseudopodia, or if the granules shift

position by internal cytoplasmic motion within relatively fixed processes. The same problem is found in dermal chromatophores of lower vertebrates (Fuchs, 1914). It is easy to prove that the pigmented epithelium in the expanded light phase separates less easily from the nervous layers than when contracted, yet this does not necessarily signify an extension of the processes as a whole; an internal pigment flow into a comparatively depleted fixed process would accomplish the same result by effecting a firmer interdigitation with the rods and cones. The observations, already cited, of isolated cells with pigment-free distal processes support the independent shifting of pigment particles, and even more significant is the full retreat of pigment in certain dark-adapted retinas, while the tapetal guanin still occupies a more distal position (Fig. 449); equally interesting are instances of a general pigment retraction in which some granules are left behind in the position of extreme expansion.

The spaces between the rods and cones are not occupied completely by the pigment cell processes. There is also a structureless, hyaline ground substance which when fresh shows a characteristically tenacious quality but becomes first slimy and then completely liquid after death.

2. *Cell inclusions:*

a. Fuscin. The most conspicuous feature of pigmented retinal epithelium is the pigment contained as cellular inclusions. It occurs in all but the albino representatives of the several vertebrate classes. Kühne (1879) long ago first offered proof that the retinal pigment is not identical with melanin of other locations, such as the chorioid; this contention, however, has been questioned by some (Miescher, 1923) who deny that a significant chemical difference has been demonstrated, while on the other hand Majima(1925) cites several workers in support of his conclusion that the retinal pigment is not a pure melanin but a lipofuscin—a pigment covered with lipoid substance. Since the pigment of the retina is brown instead of black, even in thick layers, Kühne gave it the appropriate name "fuscin." The shade varies from dark brown in most vertebrates to light brown, reddish brown and even to purple tones; Raelmann (1907) claims that the rounded granules are a deep reddish brown, while the needles tend to a lighter brownish yellow.

Unlike the pigment in some other locations, that of the retina is for the most part crystalline. The cell base is devoid of pigment except for traces about the periphery; in the body of the cell are both rounded, amorphous granules of different sizes and crystalline pigment, whereas the processes contain nothing but crystals (Fig. 434). The crystalline type prevails in fishes that frequent the light, and this pigment, located chiefly in the processes, is the more mobile. On the other hand, nocturnal fishes, or those that live in dim light, have a preponderance of the comparatively sluggish,

rounded granules. The crystalline pigment granules have characteristic shapes (Figs. 436 to 443); in man they are short (1μ to 5μ in length), and, except under high magnification, are not easily distinguished from the amorphous granules; the needle type is best represented in birds. In the cell body the pigment granules lack any definite arrangement, while the crystalline granules of the processes are arranged serially, parallel with their long axes.

The entire pigment granule consists of two components: the fuscin material itself and an albuminous ground substance to which the former is firmly bound by adsorption (Miescher, 1923). The characteristic shape of the pigment granule is a property of the bearer substance, not of the fuscin proper. Raelmann (1907) observed with the ultramicroscope that each pigment granule is embedded in an investing protoplasmic matrix which Majima (1925) interprets as a capsular sheath staining with various dyes.

Fuscin is very resistant to chemical attack; this is remarkable in view of the complete bleaching which it exhibits *in vitro* when exposed to the action of light. An intracellular consumption and replacement of pigment granules throughout life is not known to occur although Luna (1911*a*), for one, assumes it; they appear to persist as inert cell inclusions.

b. Guanin. Some nocturnal animals have a retinal tapetum quite apart from the commoner chorioidal tapetum (Kühne, 1882; Chievitz, 1889). This is true of numerous fishes (Wunder, 1925) and also of the crocodile (Abelsdorff, 1898) and alligator (Chievitz, 1889; Laurens and Detwiler, 1921). In the fish *Abramis*, for example, the retinal tapetum occupies a sector comprising the upper two-thirds of the visual field, and this region appears shining white in contrast to the lower third which is dark brown; in a detached retina the tapetal region is quite transparent. Microscopically, the retinal cells prove to be filled with many white guanin granules between which are sparsely interspersed dark ones of fuscin, but the differentiation is apparent only under reflected light. The guanin granules occur throughout the cell but chiefly in the processes where in *Abramis* they constitute a thick crust (cf. Fig. 449). The tapetum of the alligator includes the dorsal and posterior portions of the retina. About midway between optic papilla and periphery the guanin zone is broad and the epithelium is deficient in fuscin which merely forms a narrow border at its vitreal margin (Fig. 444). Above and below this level, the fuscin increases progressively and the guanin decreases (Fig. 445); close to the optic nerve, as in the remaining non-tapetal area of the retina, guanin is absent (Fig. 446).

The value of the tapetum lies in the fact that weak light, coming from the bottom, is reflected back a second time into the visual elements and so allows low intensities to be utilized. The tapetum consists of a material, called by Kühne "guanin," which is also found in the scales, argentea and other parts of fishes and collectively is said to be the primary source of the

similar product in guano. Animals with a tapetum are of further interest in that they allow the bleaching of visual purple to be observed by the naked eye (Abelsdorff, 1898).

c. Lipochrin. Fat droplets, studied extensively by Kühne (1879) and named by him "lipochrin," appear constantly in the unpigmented cell base of some animals (Fig. 434); their coloration with dyes parallels that of the oil globules in cones (Oguchi, 1914). Such droplets occur in the frog, rabbit and certain birds but are absent in the hog, ox and man. Most commonly lipochrin takes the form of a large, single drop which may even extend past the level of the nucleus; if there are several droplets, smaller ones are usually arranged about a larger. The color in the frog is golden to lemon; in some owls, yellow to orange; in other owls and the rabbit, colorless. Light gradually fades these colors.

d. Myeloidin. The base of the pigment cell also often contains myeloid, or aleuronoid, granules (Fig. 434). These are colorless colloidal bodies, spheroidal or cuboidal in shape and of wax-like luster. Originally interpreted as colorless fat, they were differentiated by Kühne (1882) and named "myeloid bodies" since their reactions most closely resemble myelin. Corroborative is the observation of Oguchi (1914) that they always show the same staining behavior as the substance of the rod's outer segment; the chemical nature of myeloidin has also been studied by Lo Cascio (1923). Aleuronoid granules have been described in amphibia, birds and mammals including monkeys, but are reported absent in man (but cf. Kreibich, 1912). Myeloid granules tend to occupy the region farthest beneath the nucleus, yet at times they fill the base and even extend past the fat droplets into the cell body.

3. *Origin of pigment:*

The labors of many investigators have amply proved that the retinal pigment does not contain iron and is not of hematogenous origin (Miescher, 1923). Nevertheless, there are many opinions as to the immediate source. Luna (1911*a*; 1912; 1913) insists that the mitochondria found at the base of the cells are antecedent to the pigment granules which are further held to be progressively consumed under the action of light; this thesis was not supported by Luna's later observations (1917) on living cultures nor by Smith's (1920) careful studies with the same method, yet such a belief is still maintained by the former. Kreibich (1912) described the myeloid bodies in whole or in part transforming into pigment granules and Luna (1912) also includes them as somehow intermediary. Von Szily (1911), like certain earlier observers, thought the pigment originates in the nucleus, but this is denied by Champy (1911), Hooker (1915) and Smith (1920). The latter two agree that pigment in general arises and develops in the cytoplasm and is not to be correlated with any definite precursor. Accord-

ing to Smith, there is first the formation of a colorless chromogen, and this stage is followed by the production of color in the chromogen.

III. THE FUNCTIONAL ACTIVITIES OF RETINAL ELEMENTS

The eye is exquisitely sensitive to light, some thirty thousand times more so than any instrument ever devised. If its limiting capacities of reception be measured in terms of the weakest to the brightest light perceived, the variation is as twenty billions is to one (Nutting, 1920); this ratio far exceeds the range of any other physical instrument. In differential sensitivity the eye appreciates changes of about $^1/_{150}$ of the total illumination.

Although pressure and electrical stimulation are capable of exciting the optic nerve fibers and causing a luminous subjective sensation, the sole adequate stimulus of the retina proper apparently is light rays between the lower red and extreme violet, which, nevertheless, elicit no response whatever except when applied to the visual cells. Moreover, the response is entirely out of proportion to the strength of the stimulus, and for this reason it is necessary to assume that the light acts upon a mechanism (somewhat comparable to a photographic plate) containing photosensitive substances which are capable of liberating potential energy and thus producing an augmented effect over the ether vibrations that act as a primary stimulant.

The exact level of the image in the retina, and, by assumption the level where the transformation from light energy to nervous excitation takes place, is not surely known. That it occurs between the chorioid and the external limiting membrane follows from classic experiments such as Heinrich Müller's, with Purkinje's figures, and there is good evidence (Brücke, 1844, 1845; Boll, 1871) that it does not lie sclerad of the lentiform apparatus; Garten (1907) has argued from experiments that the image may be about at the level of the external limiting membrane. On the other hand, Hess (1907) and Perlet (1909) contend that the hen and pigeon do not see in blue light, presumably for the reason that the red oil globules in the cones absorb the blue rays and the outer segments so remain unstimulated; this leads them to conclude that the primary locus of visual stimulation is in the outer segment.

1. *Untenable theories of vision:*

The nature of photic reception — how light vibrations are transformed into nervous impulses — is not understood. The remarkable transparency of the rods and cones, and the resultant low absorption, must decrease greatly the available stimulative power of light, because, as Draper's law states, light must be absorbed to effect chemical change. Some have viewed the transverse lamellae of the outer segments (pp. 892 and 895) as an arrangement for reflecting light back into the inner segments (and of course

the retinal tapetum when present also acts in this way); it is known that a pile of glass plates, although transparent, is a most efficient reflector. Such re-utilization of light, however, would not be useful in the registry of sharp images in bright-light vision; it is chiefly valuable in bringing diffuse, weak illumination to the receptive threshold. Absorbed light waves apparently are not transformed into heat waves which in turn become the effective excitative agents; at least, infra-red waves, focussed on the eye, do not give the sensation of light.

Some (Angelucci, 1890) have been impressed with the changes in position which many visual cells undergo under the influence of light (p. 908), and have naïvely identified these movements with the translation of photic into nervous energy. Such a photomechanical interpretation is untenable; the movements are not only too slow but also the complex visual phenomena cannot be stated in terms so crude and simple; moreover, it is certain that such changes in the visual cells are insignificant if not lacking in many animals, including mammals. If, as is commonly held, the inner segment is most sensitive to short wave lengths (but cf. Wunder, 1925), this phenomenon must be independent of color perception for the reason that the hen and pigeon do not see in blue light (Hess, 1907; Perlet, 1909).

Neither are the electric currents of the retina more than a token or accompaniment of the activity of excitable tissue. They indicate that light has stimulated the retina but do not aid in determining the really fundamental question as to how that excitation is brought about.

Retinal pigment in vitro can be bleached experimentally with light but this is scarcely proved in living animals under normal conditions. That attempts to interpret such pigment loss as the decomposition of a photosensitive material essential in the visual process are inconsequential, follows from the obvious fact that albino animals can see although they are lacking in pigment. This alone excludes the pigment as an essential visual stuff. It is possible, as Herzog (1905) suggested, that pigment expansion in light is of use in a secondary capacity by storing up radiant energy in the sense that the dark heat waves thus made available accelerate the chemical processes taking place in the photoreceptors.

Similar objections apply to any alleged significance of the bleaching of the oil globules in the visual cones, which likewise has not been demonstrated in the undisturbed living eye; furthermore, the globules seemingly are not located favorably in the cell and are absent in many animals, including mammals.

2. Photochemical theory of vision:

What, then, may the photosensitive substances be that are intermediary in the production of chemical changes which in turn give rise to nerve impulses? One such is visual purple, demonstrated by Boll (1876), studied

extensively by Ewald and Kühne (1877) and found in the outer segments of the rods of all vertebrates. It bleaches in the light and regenerates in the dark, and this latter property in particular is held to be at the basis of rod vision at low intensities; at least the greater sensitiveness parallels the accumulation of visual purple, although it is conceivable that the real factor is some concomitant chemical change. This regeneration seems to depend on a substance derived from the pigmented epithelium, as can be proved experimentally by separating and restoring the contact between the pigmented and nervous layers. Regeneration is hastened by pilocarpine and muscarin, due apparently to the increased secretory activity of the epithelium. However, the pigment granules themselves cannot be concerned because the same regenerative phenomena occur in albinos.

Since visual purple is an unstable substance, readily decomposed by light waves, it was early hoped that this would become the basis of a general physiological theory of vision. Although it seems to play an important part in the functional response of the rods, visual purple, nevertheless, lost in interest and came to be considered unessential to vision. Its reported complete absence in some animals and from the fovea of mammals has been held to preclude the basing of an inclusive theory on it. The demonstration of a photosensitive substance like visual purple, however, would make it seem probable that one or more similar compounds exist which have not been detected, due to their dilution or lack of color, but which are capable of exacting the visual apparatus. In this connection should be mentioned the results of Hecht and Williams (1922), which indicate the presence in the cones of a photosensitive substance whose behavior and absorption spectrum suggest strongly visual purple in dilute solution. This conclusion is in agreement with the opinion of Weigert (1921), and, if verified, would remove the objections to visual purple as the physical basis of an inclusive theory of vision.

The next step is to inquire how a photosensitive material might participate in the process of vision. This has been done by Weigert (1921) who developed an interesting explanation based on significant chemical analogies. Even more important is the theory of Hecht (1920; 1921*a*, *b*) who has studied the photochemistry of both invertebrate light responses and the phenomena of the human retina; many facts have led him to conclude that the photosensitive mechanism is composed of two processes: the initial one has the characteristics of a photochemical reaction, while the subsequent process is an ordinary chemical reaction which is catalyzed by the substance formed in the earlier one.

In the first reaction, a photosensitive substance, *S* (which he identifies with the visual purple), is decomposed by the action of light into two products of decomposition, *P* and *A*. This reaction is reversible and in the dark the two decomposition products, which are as well the precursor

substances of S, regenerate and unite into the original photosensitive material. The process which goes on in the sense organ may then be written:

$$S \underset{\text{"dark"}}{\overset{\text{light}}{\rightleftharpoons}} P + A$$

It is further believed that the decomposition products, $P + A$, catalyze an inert substance, L, which is changed into a chemically active substance, T, that in turn acts on the nerve to initiate the sensory impulse.

In conjunction with these conclusions it is stimulating to consider the droplets described by Kolmer (1909; 1914) and Detwiler (1923) and their behavior which suggests a causal relation to the photosensitive substance, presumably visual purple (pp. 905, 906).

3. *Functional interpretation of the visual apparatus:*

Taken as a whole, many facts point incontestably to the conclusion that the rods and cones are the sensitive photoreceptors. Among such proofs are their specialized structure, the location of the optical image at their level, and the absence of other elements in the mammalian fovea where vision is keenest. On the contrary, the pigment cell is accessory, even though it may serve other purposes than that most obviously related to its pigment.

When the component parts of the visual cells are considered, the inner segment appears to be at the level of the optical image and so is at once suggested as the locus where the light rays actually are transformed into a sensory impulse. Nevertheless, the rod's outer segment contains the photosensitive visual purple and there are reasons for suspecting that the corresponding portion of the cones is important (p. 892), so the identification of the chief level of chemical and nervous activity is by no means certain (Hess, 1907; Perlet, 1909). The myoid is of use in changing the positions of the cells in many animals in an important way that will be explained more fully in a subsequent section (p. 908). The single or compound lentiform apparatus through its appropriate shape (p. 896) can well serve to concentrate the light, perhaps diverging as it passes the focal plane, upon the outer segments, which in the rod at least is known surely to contain an important photosensitive stuff. The oil globules (especially the colorless ones) of the cones can act in the same manner and intensify the stimulation of the hypothetically dilute receptive stuff in the outer segment. The full interpretation of the colored oil globules is more involved and may best be deferred to a later paragraph (p. 911). The fibrils are easiest interpreted as neurofibrillae; that some or all may be sustentacular is quite possible and at present positive correlation with actual function is without basis.

These general interpretations will be supplemented with more detailed applications in the paragraphs that follow.

4. *The duplicity theory and correlated retinal adaptations:*

In 1866 Max Schultze advanced the "duplicity theory" of specialized function in the visual cells; this view has been advocated by others, especially by v. Kries (1894), and many facts support it. The theory holds that the visual cones are the prime receptors in distinct, bright-light vision and in color vision, while the rods are incapable of mediating color discrimination or critically sharp vision but become especially sensitive to low intensities of light (Nagel, 1905; Helmholtz, 1911; Parsons, 1915).

In support of this hypothesis may be advanced many facts: Cones alone occupy the fovea in which direct, distinct vision, as well as the highest color sensibility, exists; furthermore, most reptiles that prefer bright light, or the direct rays of the sun, have cones exclusively. On the contrary, toward the periphery of the human retina rods are abundant, while vision becomes indistinct, color discrimination is lost and sensitivity to low light intensities increases; rods are exclusively present in some nocturnal animals and almost so in others. It is worth recording that Siven (1905; 1912) objects vigorously to the denial of color perception to the rods and advances many arguments from the phenomena of color vision to substantiate his contention that the rods appreciate colors in the shorter wave lengths.

It is stimulating to analyze the positional changes in the rods, cones and retinal pigment that occur in bright and dim light and to interpret these in terms of the duplicity theory. The chief natural agent that produces demonstrable effects on the retinal pigment and visual cells is light. Although it is not at all clear that such changes are directly concerned with the visual process (because they are inappreciable or lacking in some animals, including mammals), yet the end results may well be of definite value to those individuals possessing them by enhancing the optical conditions attendant on photic reception.

The pigment distribution tends to shift in light and darkness; this has been demonstrated in many vertebrates. In fishes and anuran amphibia it is especially extensive, the heavy pigment band in some instances changing under illumination from a narrow to a broad zone (Figs. 449 and 450). In birds the migration is somewhat more restricted. In urodeles and reptiles it is demonstrable but exceedingly slight, whereas in mammals appreciable differences have not been shown.

The myoid of visual cells is the part that actively responds; its change in length may be as high as 10:1. The cone myoid of fishes, anura and birds elongates greatly in darkness and shortens in light (Figs. 449 and 450). In urodeles, reptiles and mammals the change is very slight but apparently

is appreciable. The response of the rod myoid is the exact reverse; it elongates in the light and shortens in the dark (Figs. 449 and 450). This is extensive in fishes, anura and birds but is negligible or absent in urodeles, reptiles and mammals.

It has been commonly assumed that positional changes occur in man as prominently as in lower vertebrates; this is found in numerous texts and writings and has served as the basis of much theorizing (e.g., Klein, 1911; Cajal, 1911; Bard, 1919; Cobb, 1919). On the contrary, it is doubtful whether any significant movements of either pigment or visual cells occur; if present at all, they are probably so slight as to be negligible (Arey, 1915*b*).

The easiest way to understand the relations in the several photomechanical changes is by following the course of events in typical dark-adapted and light-adapted retinas (Figs. 449 and 450). In the light phase the cone myoid shortens until the ellipsoid is brought near the external limiting membrane and both the ellipsoid and the outer segment are buried in pigment which has expanded in a broad band. In the dark phase the cone myoid elongates and the rod myoid shortens, thereby drawing the rod nearer the external limiting membrane; at the same time the pigment retreats toward the chorioid to form a compact, narrow layer.

Such positional changes are interpreted as of use in furthering efficient vision. It seems logical that the insinuation of pigmented processes between the individual visual elements effects for the latter a high degree of optical isolation by absorbing dispersed rays which would otherwise be refracted sidewise from neighboring cells, and especially from the highly refractive ellipsoids and oil drops of cones. From the standpoint of sensory reception, the individual stimulation of the visual cones is obtained and the sharpness of the retinal image is in this way enhanced. The withdrawal of the pigment in dim light might be thought of as a response which allows the visual cells to utilize all the weak light available, although at the cost of a less sharp image. Furthermore, there is good reason to believe that the cones are concerned in bright-light vision, the rods with dim-light (twilight) vision. Hence, a shortening of the less sensitive cone in bright light, drawing it down nearer the source of illumination and the image plane (p. 907), while the rod of lower threshold at the same time elongates and is thus moved out of the way into the protective pigment mantle, would appear to be a useful maneuver. The converse procedure in dim light- by which the more sensitive rods are shortened and the highly refractive and temporarily useless cones, with their lens-like ellipsoids no longer masked by pigment, are lengthened—would be fully as advantageous, for the rods are thereby brought nearer the source of illumination and so present a homogeneous layer of equal-sized and equally refractive bodies from which pigment can then safely withdraw. If there is a reflecting tapetum it is serviceable in the dark phase by assisting in the utilization of weak intensities.

These several relations can be useful only if the threshold of the visual cells and retinal pigment is sufficiently high so that the responses in weak light and darkness are identical; a differential response in total darkness alone would accomplish no useful purpose. When the matter is put to trial (Arey, 1919; cf. Wunder, 1926) it is found that the responses to light begin at about an intensity which allows bright colors to be recognized by the completely dark-adapted human eye, and the full light phase is assumed only when the illumination is increased to an intensity which permits the reading of ordinary print. These results indicate that the mechanically advantageous positions of the visual cells and pigment are indeed present for a theoretically more efficient dim-light and bright-light vision.

Further interesting correlations exist with respect to habits of life (Arey, 1916*a*; Wunder, 1925). Among fishes the highest pigment expansion is found only in those with diurnal habits; in such it becomes heaped about the retracted cones but never retreates fully in the dark. On the contrary, nocturnal fishes have pigment that exhibits maximum contraction and fails of extreme expansion. Interpreted in terms of cone vision and rod vision, these correlated responses are significant.

This adaptive mechanism is obviously useful only in those animals that have a marked set of photomechanical changes. How, then, do the eyes of other animals, and notably mammals and man, function so admirably in its absence? The asumption is that different anatomical and physiological conditions make such movements unnecessary. One might suspect that all the light rays which are brought to focus (presumably in the inner segment of the visual cells of mammals) undergo total reflection in the outer segment and so do not pass laterally to stimulate neighboring cells, whereas in lower vertebrates a part of the rays is lost, due to lower refractivity, and so would pass into neighboring elements if it were not for the intervening pigment. However, in the outer segment of the rod of representative lower and higher vertebrates the optical conditions prove to be such that total internal reflection occurs about equally (Garten, 1907); for this reason the supposition that in lower animals the pigment primarily compensates for a lack of refractivity of the substance of the rod's outer segment is without support. Lateral losses from the cones are presumably more serious due to their shape and composition, and it seems reasonable that the pigment does serve to absorb stray rays from both elements.

For explanations as to the compensations for the absence of marked movements in the pure rod retina (e.g., shark), pure cone retina (e.g., reptile) or the interesting urodele retina, one may consult the detailed applications in Garten (1907, pp. 103–111). These are at least stimulative, even if carried well into the speculative realm.

The absence of significant movements in man and mammals, the different morphology of cones at the center and periphery, and the sparseness

of pigment can be explained as follows: The cones in the central regions of the retina are slender and their long outer segments taper but little and reach almost as high as the rods. This similarity in structure makes a nearly homogeneous field. Furthermore, a typical ellipsoid is replaced by the "fiber apparatus" and in all mammals but marsupials the oil drop is lacking. So it appears that light entering the rods and cones is largely retained and escapes chiefly into the low pigment cells at the tips. Lateral escape is nearly as well safeguarded as in a pure rod retina. Farther toward the periphery conditions are less favorable; the outer segments of the cones are conical and their ends lie at a lower level, while the inner segments and fiber apparatus are plumper. These relations are more conducive to a lateral loss of light, and the stimulation of neighboring rods is thereby possible. Is it that our indistinct vision in these regions is due to this image confusion through form changes? Moreover, as the cone becomes slenderer and more rod-like in shape toward the fovea, is it also possible that the rod functions are additionally assumed by them? In other words, cones nearer the periphery may be chiefly for color vision but as the outer segment elongates, their sensibility to colorless stimuli increases.

5. Color vision:

So little is definitely known about the mechanism of color vision that none of the popular theories of its method of action can be considered too seriously. They are explanations designed to fit certain data, and this they do somewhat incompletely and inadequately. Each is an ingenious possible solution and yet all may fall quite short of the true expression of the actual process.

There is good reason to consider the cones as the elements responsible for color vision (p. 895). Of these the colored oil globules may have a functional significance in color perception. They are present in reptiles and birds but are best developed in birds with diurnal habits; in the latter Waelchli (1883) was able to distinguish eight kinds of colored globules. These occupy the full breadth of the inner segment and so can serve as differential filters which absorb certain rays and permit others to act on the presumptive unstable photosensitive substance of the outer segment (p. 895). Even if this sensitive material is identical in different cones, each kind of cone could be stimulated by differential light rays and so the basis for a mechanism of color vision in these color-sensitive elements would be present. In this regard one naturally compares the operations of photolithography or the methods of producing photographs in natural colors, such as the process of Lumière.

There are no globules in mammalian cones, but these are unnecessary if there is an increase in the specificity to different wave lengths of the receptive substances in the cones so that there can be differential stim-

ulation. This would represent a higher stage in color-differentiating mechanisms.

6. Miscellaneous activities:

There are many additional observations on the activities of the retinal pigment and visual cells, but many of them as yet throw no definite light on the sensory phenomena of reception. Some seem to be capable of interpretation only on the basis of generalized protoplasmic responses to definite adequate stimuli: especially is the similarity between the physiology of retinal pigment and that of dermal chromatophores significant (Arey, 1916*a*). Certain stimulating agents are effective that are purely laboratory impositions and never are met with in the natural environment of the animal.

The time necessary to complete the several photomechanical movements is of interest on account of their astonishing sluggishness. In fishes and the anura, for example, the adaptation times of the retinal pigment and visual cells in changing between the extremes of light and darkness, and the reverse, occupy a half hour or more (Arey, 1916*a*). This is especially surprising in comparison, for example, with the rapid dermal color changes in cephalopods, fishes, amphibia and reptiles.

Temperature has interesting effects upon the position of the retinal pigment and visual cells (Arey, 1916*a*). In fishes the action is directly upon the protoplasm of the cells themselves. In the frog the results on the pigment are of quite a different sort and indicate a nervous control brought into action by temperature. The possibility of a hormone influence in life does not hold; of all the endocrin secretions adrenalin alone causes pigment to expand, but the characteristic temperature reactions continue even after the suprarenals are removed (unpublished observations). Experiments on excised eyes (Arey, 1916*a*) show in general that the characteristic movements to light and temperature occur in fishes, but fail in the frog's pigment, although improved results for the latter are claimed under the most favorable nutritive conditions (Kumagai, 1916).

Carbon dioxide is an efficient anesthetic which can be proved to act directly on the visual cells and retinal pigment to arrest them and prevent further movements regardless of the subsequent treatment. Although Dittler's (1907) theory of the chemical stimulation of the cone myoid of the frog is logical for that animal, it does not meet the conditions in fishes as experiments with anesthetics and temperature prove (Arey, 1916*a*).

Several experimenters since the time of Englemann (1885) reported the corroboration of his finding that the illumination of one eye causes the characteristic responses in the other, but this is apparently not so (Fick, 1891; Fujita, 1911). Furthermore, Fujita (1911) and the writer (1916*a*) have failed to confirm his further contention that illumination of the photo-

sensitive skin induces positional changes in the retina of frogs whose eyes remain covered, and consequently disprove an interrelation between the integumentary photoreceptors and retinal elements through retino-motor nerves. On the contrary, the writer has recorded elsewhere (1916*b*) experimentation which throws some light on the function of the efferent fibers known to exist in the optic nerve.

7. Adaptations to life habits:

Wunder (1925, 1926) studied the visual and pigment cells of fishes in relation to habits of life and found interesting and important correlations in the fishes with diurnal habits that live in bright light near the surface, and those that live in dim light due to depth or a muddy, weedy habitat, or have frankly nocturnal habits.

The rods of bright-light fishes are large, robust and not abundant (Fig. 399). Dim-light fishes have extremely small and slender rods in great numbers that ofttimes lie at different levels and apparently are most effective in making the best of weak stimuli (Fig. 400); their small size and abundance would conspire for the rendering of sharper images. The only exception is in dim-light fishes with poorly developed eyes where the rods are large and sparse (Figs. 499 and 450).

Contrary to what might theoretically be expected, the cones of both groups are long and robust. Apparently, then, the dim-light habitués demand well-functioning cones (Fig. 416); only the dim-light fishes with poorly developed eyes (like the bull head) have small cones reduced in number (Fig. 417). The visual elements of amphibia exhibit the same type of structure as in fishes with poorly developed eyes (Figs. 402 and 420); the number of visual elements is increased, but they are far behind the number and fineness of rods in fishes with well-developed eyes. The rod and cone layer is much thicker in typical dim- than in bright-light fishes and it is only in the former that the fullest possible extension of cones occurs (Figs. 447 and 448).

The frequency and size of cones do not differ in the upper and lower regions of the retina. Rods likewise maintain the same size in both regions but their number above is greater—even to 50 per cent in the dim-light animals, whereas the frequency is more nearly the same in bright-light fishes. This increase in the rods of dim-light fishes is responsible for the greater thickness of the retina above and is useful in utilizing the reflected light which comes mostly from below. In the upper part of the retina the ratio of cones to rods in bright-light fishes is 1:4.5, in fishes that habituate both bright- and dim-light, 1:8.4, and in dim-light fishes, 1:23.2.

The pigment of the bright-light fishes is more of the needle type and it expands maximally until heaped near the external limiting membrane, but it never contracts completely (Fig. 447). In dim-light fishes there is more

amorphous granular pigment and contraction is complete, but the light phase is more evenly diffuse (p. 910; Fig. 448). Extreme expansion and contraction prove to be mutually exclusive in the same animal (Arey, 1916*a*; Wunder, 1925). These differences are seen also in the upper and lower regions of the same retina; the lower retina in bright-light fishes has extreme expansive capability, whereas the more granular pigment above is relatively sluggish; cones likewise are more sensitive to weak light in the lower part.

In summary, dim-light fishes have smaller, slenderer rods in greater abundance than do the bright-light animals; further, the complete contraction of pigment is found. The ratio of rods to cones in these animals is also greater in the upper part of the retina which is thereby thicker and may contain a reflecting guanin layer. All these factors are favorable to the utilization of the weak light which comes mostly from below to fall on this region of the retina. The picture in bright-light fishes is of a thinner retina with relatively more cones and a more abundant pigment that expands maximally, thereby isolating these stronger stimulated elements. Similar adaptations are seen also in the upper and lower regions of the same retina of some fishes.

The present conception of the physiology of the visual cells and retinal pigment is admittedly incomplete and in many respects highly unsatisfactory, yet, even so, more is probably known of the eye as a working mechanism than of other sense organs. The interpretation of any sensory process is inherently elusive because of the intangible factors involved, and this will perhaps always necessitate more speculation and hypothesizing than might be wished. Besides the fundamental facts already well established and correlated, there is a considerable body of solid observation and experiment as yet uncoordinated and so for the moment not of especial significance; much of this, however, is potentially valuable and will at the proper time assume a place of importance in the scheme of retinal physiology. A renewal of the attack, both cytological and experimental, with the most modern methods utilized and extended, promises much. In view of what has been accomplished in other fields, as, for example, the correlated efforts of cytology and genetics, one does not feel undue temerity in prophesying the inevitable discovery of the retinal Rosetta stone.

IV. BIBLIOGRAPHY

Abelsdorff, G. 1898. Physiologische Beobachtungen am Auge der Krokodile. *Arch. f. Anat. u. Physiol.*, Physiol. Abt., Jahrg., 155.

Angelucci, A. 1878. Histologische Untersuchungen über das retinale Pigmentepithel der Wirbeltiere. *Arch. f. Anat. u. Physiol.*, Physiol. Abt., Jahrg., 353.

——— 1890. Untersuchungen über die Sehthätigkeit der Netzhaut und der Gehirns. *Untersuch. zur Naturlehre d. Menschen u. d. Thiere (Moleschott)*, 14, 231.

Arey, L. B. 1915*a*. The occurrence and the significance of photomechanical changes in the vertebrate retina—an historical survey. *J. Comp. Neurol.*, **15**, 535.

——— 1915*b*. Do movements occur in the visual cells and retinal pigment of man? *Science*, N. S., **42**, 915.

——— 1916*a*. The movements in the visual cells and retinal pigment of the lower vertebrates. *J. Comp. Neurol.*, **26**, 121.

——— 1916*b*. The function of the efferent fibers of the optic nerve of fishes. *Ibid.*, **26**, 213.

——— 1916*c*. Changes in the rod-visual cells of the frog due to the action of light. *Ibid.*, **26**, 429.

——— 1919. A retinal mechanism of efficient vision. *Ibid.*, **30**, 343.

Bard, L. 1919. Du rôle des bâtonnets et des pigments rétiniens dans la perception des formes et des couleurs. *J. de physiol. et de path. gén.*, **18**, 276.

Bernard, H. M. 1903. Studies in the retina: rods and cones in the frog and in some other amphibia. *Quart. J. Micr. Sci.*, N. S., **43**, 23.

Boll, F. 1871. Beiträge zur physiologischen Optik. *Arch. f. Anat. Physiol. u. wiss. Med.*, Jahrg., 530.

——— 1876. Zur Anatomie und Physiologie der Retina. *Monatsber. Berlin. Akad.*, Jahr. 783.

Brammertz, W. 1914. Über das normale Vorkommen Glycogen in der Retina. *Arch. mikr. Anat.*, **86**, 1.

Brücke, E. 1844. Über die physiologische Bedeutung der stabförmigen Körper und der Zwillingszapfen in den Augen der Wirbeltiere. *Arch. f. Anat. Physiol. u. wiss. Med.*, Jahrg., 444.

——— 1845. Anatomische Untersuchungen über die sogenannten leuchtenden Augen bei den Wirbeltieren. *Ibid.*, Jahrg., 387.

Cajal, S. R. 1894. *Die Retina der Wirbelthiere.* Wiesbaden: Bergmann.

——— 1898. (See Greeff, 1898.)

——— 1911. *Histologie du système nerveux de l'homme et des vertébrés.* Paris: Maloine.

Cameron, J. 1905. The development of the retina in amphibia. *J. Anat. and Physiol.*, **39**, 135; 332; 471.

1911. Further researches on the rods and cones of the vertebrate retina. *Ibid.*, **46**, 45.

Cattaneo, D. 1922. La struttura della retina nei vertebrati. *Ann. di ottal. e clin. ocul.*, N.S., **5**, 349.

Champy, C. 1911. Recherches sur l'absorption intestinal et le rôle des mitochondries dans l'absorption et la sécrétion. *Arch. d'anat. micr.*, **13**, 55.

——— 1914. Note de biologie cytologique. Quelques résultats de la méthode de culture des tissus. IV. La rétine. *Arch. de zool. expér. et gén.*, **55**, 1.

Chievitz, J. H. 1889. Untersuchungen über die Area centralis retinae. *Arch. f. Anat. u. Entwickl.*, Jahrg. (Suppl. Bd.), 139.

Chittenden, R. H. 1879. Beiträge zur Histochemie des Sehepithels. *Unters. physiol. Inst. d. Univ. Heidelberg*, **2**, 438.

Cobb, P. W. 1919. Dark-adaptation with especial reference to problems of night-flying. *Psychol. Rev.*, **26**, 428.

Detwiler, S. R. 1923. Studies on the retina. An experimental study of the gecko retina. *J. Comp. Neurol.*, **36**, 125.

Detwiler, S. R., and Laurens, H. 1921. Studies on the retina. Histogenesis of the visual cells in Amblystoma. *Ibid.*, **33**, 493.

Dittler, R. 1907. Über die Zapfenkontraktion an der isolierten Froschnetzhaut. *Arch. f. d. gesam. Physiol.*, **117**, 295.

Dobrowolsky. 1871. Zur Anatomie der Netzhaut. *Arch. f. Anat., Physiol. u. wiss. Med.*, Physiol Abt., Jahr., 221.

Engelmann, T. W. 1885. Über Bewegungen der Zapfen und Pigmentzellen der Netzhaut unter dem Einfluss des Lichtes und des Nervensystems. *Arch. f. d. gesam. Physiol.*, **35**, 498.

Ewald, A., and Kühne, W. 18--. Untersuchungen über den Sehpurpur. *Unters. a. d. physiol. Inst. d. Univ. Heidelberg*, **1**, 139.

Fick, A. 1891. Untersuchungen über die Pigmentwanderung in der Netzhaut des Frosches. *Arch. f. Ophth.*, **37**, Abt. 2, 1.

Flemming, W. 1898. Über das Fehlen einer Querschichtung in den Kernen der menschlichen Stäbchenzellen. *Arch. f. mikr. Anat. u. Entwickl.*, **51**, 704.

Fritsch, G. 1904. Die Retina-elemente und die Dreifarbentheorie. *Abhandl. d. k. preuss. Akad. d. Wissensch.*, Jahr., Abt. 2, Berl.

——— 1911*a*. Der Ort des deutlichen Sehens in der Netzhaut der Vogel. *Arch. mikr. Anat.*, **78**, 245.

——— 1911*b*. Nachtrag. *Ztschr. f. wiss. Zool.*, **100**, 160.

Fuchs, R. F. 1914. Der Farbenwechsel und die chromatische Hautfunktion der Tiere. In Winterstein, *Handbuch der vergl. Physiol.*, **3** (Hälfte 1, Teil 2). Jena: Fischer.

Fürst, C. M. 1904. Zur Kenntnis der Histogenese und des Wachstums der Retina. *Lunds Universitets Ars-skrift*, **40**, 1.

Fujita, H. 1911. Pigmentbewegung und Zapfenkontraction im Dunkelauge des Frosches bei Einwirkung verschiedener Reize. *Arch. f. vergleich. Ophthal.*, **2**, 164.

——— 1921*a*. Mikrochemische Verschiedenheit in der Netzhaut des Dunkel- und Hellfrosches. *Chuwogankaibo*, **13**. (See Majima, 1925.)

——— 1921*b*. Verschiedene Färbung der Sehzellen (Demonstration). *Nippon-gankagakkai-Zasshi*, **25**. (See Majima, 1925.)

Garten, S. 1907. Die Veränderungen der Netzhaut durch Licht. In Græfe-Saemisch, *Handbuch der gesamten Augenheilkunde*. Aufl. 2, Bd. 3, Kap. 12, Anhang. Leipzig: Englemann.

Greeff, R. 1898. R. Cajal's neuere Beiträge zur Histologie der Retina. *Ztschr. f. Psychol. u. Physiol. d. Sinnesorg.*, **16**, 161.

——— 1900. Die mikroskopische Anatomie des Sehnerven und der Netzhaut. In Graefe-Saemisch, *Handbuch der gesamten Augenheilkunde*. Aufl. 2, Bd. 1, Kap. 5. Leipzig: Englemann.

Hecht, S. 1920. The dark adaptation of the human eye. *J. Gen. Physiol.*, **2**, 499.

——— 1921*a*. The photochemistry of the sensitivity of animals to light. *Science*, N.S., **53**, 347.

——— 1921*b*. The nature of foveal dark adaptation. *J. Gen. Physiol.*, **4**, 113.

Hecht, S., and Williams, R. E. 1922. The visibility of monochromatic radiation and the absorption spectrum of visual purple. *J. Gen. Physiol.*, **5**, 1.

Held, H. 1904. Zur weiteren Kenntnis der Nervenendfüsse und zur Struktur der Sehzellen. *Abhandl. d. math-phys. Kl. d. k. Sachs. Gesell. d. Wissensch.*, **29**, 145.

Helmholtz, H. von. 1911. *Handbuch der physiologischen Optik*. Aufl. 3, Bd. 2. Hamburg and Leipzig: Voss.

Herzog, H. 1905. Experimentelle Untersuchung zur Physiologie der Bewegungsvorgänge in der Netzhaut. *Arch. f. Anat. u. Physiol.*, Physiol. Abt., Jahrg., 413.

Hess, C. 1907. Untersuchungen über Lichtsinn und Farbensinn der Tagvögel. *Arch. f. Augenheilk.*, **57**, 317.

Hesse, R. 1904. Über den feinern Bau der Stäbchen und Zapfen einiger Wirbeltiere. *Zool. Jahrb.*, **7**, (Suppl.), 471.

Hooker, D. 1915. The rôles of nucleus and cytoplasm in melanin elaboration. *Anat. Record*, **9**, 393.

STANFORD UNIVERSITY LIBRARY

Howard, A. D. 1908. The visual cells in vertebrates, chiefly in Necturus maculosus *J. Morph.*, **19**, 561.

Johnson, G. L. 1896. Observations on the macula lutea. *Arch. of Ophthal.*, **25**, 1.

Kerr, J. G. 1919. *Textbook of embryology*. Vol. **2**, London: Macmillan.

Klein, F. 1911. Das Pigmentepithel der Retina. *Arch. f. Anat. u. Physiol.*, Physiol. Abt., Jahrg., 339.

Kolmer, W. 1904. Über ein Strukturelement der Stäbchen und Zapfen der Froschretina. *Anat. Anz.*, **25**, 102.

——— 1909. Über einen sekretartigen Bestandteil der Stäbchen-zapfenschicht der Wirbeltier-retina. *Arch. f. d. ges. Physiol.*, **129**, 35.

——— 1914. Zur Histologie der Augenhäute. *Anat. Anz.*, **47**, 417.

——— 1925. Bemerkungen über Adaptationsvorgänge in den Sehelementen. *Arch. f. Ophthal.*, **115**, 310.

Kotschetow, N. A. 1908. Untersuchungen über das Pigmentepithel der Retina im Zusammenhang mit der Frage über die Zellenteilung. *Arbeit. d. k. St. Petersb. Ges. d. Naturf.*, **39**, 134. (Abstract in *Ergebn. d. Anat. u. Entwickl.*, **20**, 212.)

Krause, W. 1892. Die Retina. (Fische, Amphibien.) *Internat. Monatschr. f. Anat. u. Physiol.*, **9**, 150.

——— 1893. Die Retina. (Amphibien, Reptilien.) *Ibid.*, **10**, 12.

Kreibich, K. 1912. Zur Entstehung der Retinapigments. *Berlin, klin. Wochenschr.*, **49**, 385.

v. Kries, J. 1894. Die Gesichtsempfindungen. In Nagel, *Handbuch der Physiologie des Menschen*, **3**, Braunschweig: Vieweg und Sohn.

Krückmann, E. 1899. Anatomisches über die Pigmentepithelzellen der Retina. *Arch. f. Ophthal.*, **47**, 644.

Kühne, W. 1879. Chemische Vorgänge in der Netzhaut. In Hermann, *Handbuch der Physiologie*, **3** (Teil 1). Leipz.: Vogel.

——— 1882. Fortgesetzte untersuchungen über die Retina und die Pigmente des Auges. *Unters., a. d. physiol. Inst. d. Univ. Heidelberg*, **1**, 89.

Kuhnt, H. 1877. Zur Kenntnis des Pigmentepithels. *Centralbl. f. d. med. Wiss.*, **15**, 337.

Kumagai, N. 1916. Zur Kenntnis der Bewegungsvorgänge in der Netzhaut. *Mitteil. d. med. Fakultät d. k. Univ. zu Tokyo*, **16**, 137.

Laurens, H., and Detwiler, S. R. 1921. Studies on the retina. The structure of the retina of Alligator mississippiensis and its photomechanical changes. *J. Exper. Zool.*, **32**, 207.

Leboucq, G. 1909. Contribution à l'étude de l'histogenèse de la rétine chez les mammifères. *Arch. d'anat. micros.*, **10**, 555.

Leplat, G. 1913. Les plastosomes des cellules visuelles et leur rôle dans la différentiation des cônes et des bâtonnets. *Anat. Anz.*, **45**, 215.

Levi, G. 1901. Osservazioni sullo sviluppo dei coni e bastoncini della retina degli Urodeli. *Lo Sperimentale, Arch. di biol. norm. e patol.*, **54**, 521.

——— 1914. Ulteriori studî sullo sviluppo delle cellule visive negli anfibi. *Anat. Anz.*, **47**, 192.

Lo Cascio, G. 1923. Sulla fine constituzione della retina. Ricerche chimiche e citologiche. *Ann. di ottal. e clin. ocul.*, N.S., **6**, 653.

Luna, E. 1911*a*. Ricerchi istologische sugli epitheli. L'apparato mitochondriale nelle cellule dell'epitelio pigmentato della retina. *Arch. di anat. pat. e sc. affini*, **6**, fasc. 2.

——— 1911*b*. Ricerche istologiche e istochimiche sulla retina dei vertebrati. *Monitore zool. ital.*, **22**, 119.

——— 1912. L'apparato mitocondriale nelle cellule dell'epitelio pigmentato della retina. *Arch. f. Zellforsch.*, **9**, 41.

LANE LIBRARY. STANFORD UNIVERSITY

Luna, E. 1913. Nuove ricerche sulla biologia del condrioma. (Condriosomi e pigmentato retinico.) *Anat. Anz.*, **43**, 56.

——— 1917. Note citologiche sull'epithelio pigmentato della retina coltivato "in vitro." *Arch. ital. di anat. e embryol.*, **15**, 542.

Magitot, A. 1910. Étude sur le développement de la rétine humaine. *Ann. d'oculist*, **143**, 241.

Majima, K. 1925. Studien über die Struktur der Sehzellen und der Pigmentepithelzellen der Froschnetzhaut. *Arch. f. Opbtbal.*, **115**, 286.

Mawas, J. 1910. Notes cytologiques sur les cellules visuelles de l'homme et de quelques mammifères. *Compt. rend. l'assoc. d. anat.*, réun. **12**, 113.

Miescher, G. 1923. Die Pigmentgenese im Auge nebst Bemerkungen in die Natur des Pigmentkorns. *Arch. f. micr. Anat.*, **97**, 326.

Moroff, T. 1922. Cyto-histogenese und Bau der Stäbchen und Zapfen der Retina bei Anuren. *Anat. Anz.*, **55**, 316.

Nagel, W. 1905. *Handbuch der Physiologie des Menschen.* Bd. 3. Braunschweig: Vieweg und Sohn.

Nutting, P. G. 1920. The reactions of the retina to light. *Am. Jour. Physiol. Optics*, **1**, 142.

Oguchi, C. 1914. Studien über die chemische Reaktion der Froschnetzhaut und die Pigmentwanderung am neuroepithel derselben, nebst Bemerkungen über die Farbungmethoden der Sehzellen. *Nippongankagakkai-Zassbi*, 18. (See Majima, 1925.)

Osawa, G. 1898. Beiträge zur Lehre von den Sinnesorganen der Hatteria punctata. *Arch. f. mikr. Anat.*, **52**, 268.

Parsons, J. H. 1915. *An introduction to the study of colour vision.* N. Y.: G. P. Putnam's Sons.

Perlet, G. 1909. Über den Einfluss des Lichtes auf die Netzhautelemente der Taube. *Ztschr. f. Biol.*, N. F., **52**, 305.

Pütter, A. 1912. *Organologie des Auges.* Leipzig: Englemann.

Raelmann, E. 1907. Zur Anatomie und Physiologie des Pigmentepithels der Netzhaut. *Ztschr. f. Augenheilk.*, **17**, 1.

Retzius, G. 1905. Zur Kenntnis vom Bau der Selachier-Retina. *Biol. Untersuch.*, N.F., **12**, 55.

Ritter, C. 1859. Über den Bau der Stäbchen und äusseren Endigungen der Radialfasern an der Netzhaut des Frosches. *Arch. f. Opbtbal.*, **5**, 101.

——— 1891*a*. Zur Histologie der Zapfen der Fischretina. *Internat. Monatscbr. f. Anat. u. Physiol.*, **8**, 128.

——— 1891*b*. Studien über die Stäbchenschicht der Vögel. *Ibid.*, **8**, 241.

Schaper, A. 1899. Bemerkung zur Structur der Kerne der Stäbchen-Sehzellen der Retina. *Anat. Anz.*, **15**, 534.

Schneider, K. C. 1902. *Lehrbuch der vergleichenden Histologie der Tiere.* Jena: Fischer.

Schultze, M. 1866. Zur Anatomie und Physiologie der Retina. *Arch. f. mikr. Anat.*, **2**, 175.

——— 1867. Über Stäbchen und Zapfen der Retina. *Ibid.*, **3**, 215.

Seefelder, R. 1910. Beiträge zur Histogenese und Histologie der Netzhaut, des Pigmentepithels und des Sehnerven. *Arch. f. Opbtbal.*, **73**, 419.

—— 1914. In Bach u. Seefelder, *Atlas zum Entwicklungsgeschichte des menschlichen Auges.* Leipz. and Berlin: Englemann.

Siven, V. O. 1905. Studien über die Stäbchen und Zapfen der Netzhaut als Vermittler von Farbenempfindungen. *Skandin. Arch. f. Physiol.*, **17**, 306.

——— 1912. Die Stäbchen als farbenperzipierende Organe. *Arch. f. Augenheilk.*, **71**, 157.

Smith, D. T. 1920. The pigmented epithelium of the embryo chick's eye studied *in vivo* and *in vitro*. *Johns Hopkins Hosp. Bull.*, **31**, 239.

Steinlin. 1868. Über Zapfen und Stäbchen der Retina. *M. Schultze's Arch.*, **4**, 10.

v. Szily, A. 1911. Uber die Entstehung des melanotischen Pigmentes im Auge der Wirbeltierembryonem und in Choroidealsarkomen. *Arch. f. mikr. Anat.*, **77**, 87.

Tschermak, A. 1898. Über die Bedeutung der Lichtstärke und des Zustandes des Sehorgans für farblose optische Gleichungen. *Arch. f. d. ges. Physiol.*, **70**, 297.

Van der Stricht, O. 1922. La structure de la rétine: la membrane limitante externe et les parties constituantes voisines. *Compt. rend. Soc. d. biol.*, **86**, 266.

Verhoeff, F. H. 1903. A hitherto undescribed membrane of the eye and its significance. *Roy. Lond. Opbtbal. Hosp. Reports*, **15** (Pt. 4), 309.

Waelchli, G. 1883. Zur Topographie der gefärbten Kugeln der Vogelnetzhaut. *Arch. f. Opbtbal.*, **29**, 205.

Weigert, T. 1921. Ein photochemisches Modell der Retina. *Arch. f. d. ges. Physiol.*, **190**, 177.

Wunder, W. 1925. Physiologische und vergleichend-anatomische Untersuchungen an der Knochenfischnetzhaut. *Ztschr. f. vergl. Physiol.*, **3**, 1.

——— 1926. Die Bedeutung des Adaptationszustandes für das Verhalten der Sehelemente und des Pigmentes in der Netzhaut von Knochenfischen. *Ibid.* **3**, 595.

PLATE I

Visual rods of representative vertebrates. To facilitate exact comparisons each is drawn to a uniform magnification of 1100. The various parts (outer segment, ellipsoid, paraboloid, myoid, etc.) may be identified by a uniform scheme of representation. All rods are drawn in the shortened or retracted condition typical of darkness. The figures are in part original and in part adapted or combined from various monographs.

FIG. 399.—Rod of a diurnal fish, the pike (*Esox lucius*), illustrative of the larger and sparser rods characteristic of bright-light vision. (*o.s.* outer segment; *i.p.* intermediate plate; *i.s.* inner segment; *l.b.* lentiform body; *my.* myoid; *e.l.m.* external limiting membrane; *nu.* nucleus.)

FIG. 400.—Rod of a nocturnal fish, the eel pout (*Lota vulgaris*), illustrative of the small, abundant rods characteristic of dim-light vision.

FIG. 401.—Rod of a urodele amphibian, the mud puppy (*Necturus maculosus*). The ellipsoid-paraboloid system (*el.*; *pa.*) is well shown as are the fine peripheral fibrils (*fi.*) described by Howard and others.

FIG. 402.—Red rod of an anuran amphibian, the leopard frog (*Rana pipiens*). The outer segment exhibits the superficial spiral grooving (*s.g.*) seen in many animals.

FIG. 403.—Green rod of an anuran amphibian, the leopard frog (*Rana pipiens*). The myoid (*my.*) of the inner segment is characteristically long.

FIG. 404.—Rod of a reptile, the alligator (*Alligator mississippiensis*).

FIG. 405.—Double rod of a reptile, the lizard (*Gecko swinhonis*). The outer segments show a rich content of granular striated material (*g.s.*) and a few secretion droplets (*s.d.*) formed from it (Kolmer; Detwiler); the condition shown is characteristic of light adaptation. The lentiform system contains both ellipsoid (*el.*) and paraboloid (*pa.*).

FIG. 406.—Single rod of a reptile, the lizard *Gecko swinhonis*. The condition represented is characteristic of dark adaptation; the granular substance (*g.s.*) has largely disappeared and the secretion droplets (*s.d.*) are more plentiful. Both ellipsoid (*el.*) and paraboloid (*pa.*) are prominent.

FIG. 407.—Rod of a bird, the sparrow (*Passer domesticus*).

FIG. 408.—Rod of a bird, the pigeon (*Columbia livia*).

FIG. 409.—Rod of a mammal, the brown rat (*Mus norvegicus*). A filamentous specialization of the rod characteristic of the rat and mouse.

FIG. 410.—Rod of a mammal, the hog (*Sus scrofa*). The short, slender outer segment and the relative proportions of the two segments are unusual.

FIG. 411.—Entire rod visual cell of a mammal, man (*Homo sapiens*). From the fundus, outside the macula lutea. *fi.* coarse fibril with diplosome described by Fürst, Kolmer, Held and Retzius; *o.s.* outer segment; *f.a.* fiber apparatus; *i.s.* inner segment; *e.l.m.* external limiting membrane; *nu.* nucleus; *r.f.* rod fiber; *e.k.* end knob.

FIG. 412.—Transverse section through the rod of a urodele amphibian, the mud puppy (*Necturus maculosus*), at the level of the paraboloid. Peripherally there are dots (*fi.*) which represent sections of the fine fibrils described by Howard and others.

FIG. 413.—Outer segment of a human rod, showing by swelling and bending the onset of degeneration that occurs promptly in the fresh condition.

FIG. 414.—Outer segment of a human rod showing the separation into plates that follows the stage of Fig. 413. At the right is a detached plate viewed on the flat; the scalloped rim corresponds to the superficial grooves in optical section; the darker center represents the internal core.

FIG. 415.—Outer segment of a human rod showing droplet degeneration as induced by heat, water or imperfect fixation.

PLATE I

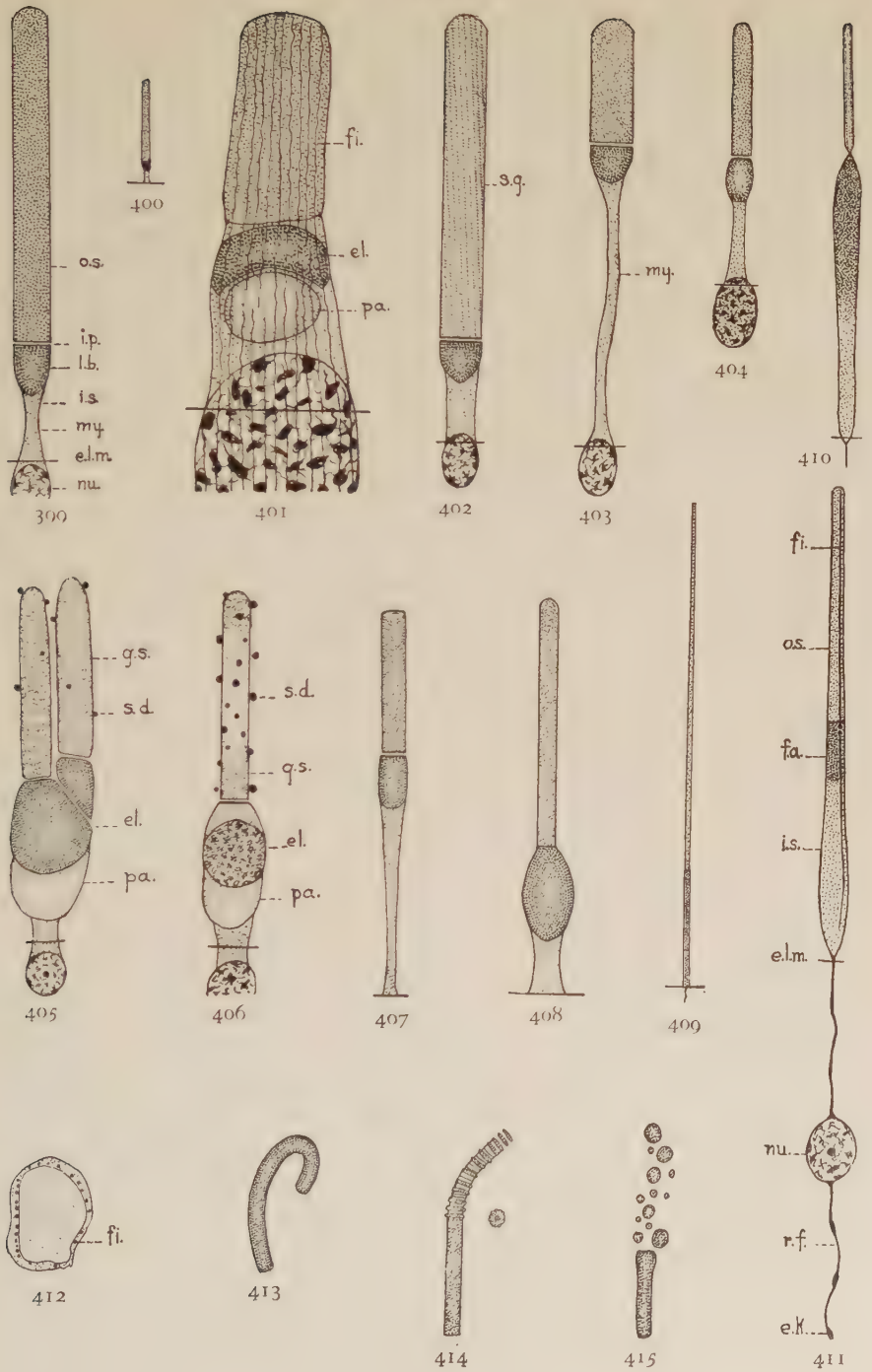

o.s.
i.p.
l.b.
i.s.
my.
el.m.
nu.
300
400
fi.
el.
pa.
401
s.g.
402
my.
403
404
410
g.s.
s.d.
el.
pa.
405
s.d.
g.s.
el.
pa.
406
407
408
409
fi.
o.s.
fa.
i.s.
el.m.
nu.
r.f.
e.k.
411
fi.
412
413
414
415

PLATE II

Visual cones of representative vertebrates. To facilitate exact comparison each is drawn to a uniform magnification of 100. The various parts (outer segment, ellipsoid, paraboloid, myoid etc.) may be identified by a uniform scheme of representation. All cones are drawn in the shortened or retracted condition typical of light. The figures are in part original and in part adapted or combined from various monographs.

Fig. 416.—Cone of a nocturnal fish, the half bream (*Blicca bjoerna*). Robust cones are characteristic of well developed eyes of both bright-light and dim-light fishes. o.s. Outer segment; l.b. lentiform body; i.s. inner segment; my. myoid; e.l.m. external limiting membrane.

Fig. 417.—Cone of a nocturnal fish, the stone grig (*Cobitis taenia*). Small, sparse cones are characteristic of those dim-light fishes with poorly developed eyes.

Fig. 418.—Twin cone of a diurnal fish, the perch (*Perca fluviatilis*). Such equal combined elements occur only in animals with well developed eyes.

Fig. 419.—Cone of a urodele amphibian, the mud puppy (*Necturus maculosus*). The ellipsoid-paraboloid system is well shown (el.; pa.) as are the fine peripheral fibrils (fi.) described by Howard and others.

Fig. 420.—Cone of an anuran amphibian, the leopard frog (*Rana pipiens*). The oil globule (o.g.), common also in the reptiles and birds, is a conspicuous feature. Spiral fibrils (s.f.) described by Hesse and others, are included.

Fig. 421.—Double cone of an anuran amphibian, the leopard frog (*Rana pipiens*). The chief (or far) cone (c.c.) is of the ordinary type; the accessory (or near) cone (a.c.) is modified.

Fig. 422.—Cone of a diurnal reptile, the horned toad (*Phrynosoma cornutum*). Besides the oil globules (o.g.) there is a refractive paraboloid (pa).

Fig. 423.—Cone of a nocturnal reptile, the alligator (*Alligator mississippiensis*). Both ellipsoid (el.) and paraboloid (pa.) are represented.

Fig. 424.—Double cone of a diurnal reptile, the Chinese lizard, (*Eremias argus*). The chief (or far) cone possesses an oil globule (o.g.) and ellipsoid (el.); the accessory (or near) cone has an ellipsoid (el.) and paraboloid (pa.) but lacks the oil globule.

Fig. 425.—Cone of a bird, the pigeon (Columba livia). The oil globule (o.g.) is highly developed, some eight varieties being recognized. The filamentous outer segment (o.s.) is likewise remarkable.

Fig. 426.—Cone of a bird, the sparrow (*Passer domesticus*).

Fig. 427.—Entire cone visual cell of a mammal, man (*Homo sapiens*). From near the ora serrata.

Fig. 428.—Entire cone visual cell of a mammal, man (*Homo sapiens*). From the fundus, outside the macula lutea. o.s. outer segment; fi. peripheral fibril described by Kolmer and Held. l.b. lentiform body; i.s. inner segment; e.l.m. external limiting membrane; nu. nucleus; c.f. cone fiber; ft. cone foot.

Fig. 429.—Cone of a mammal, man (*Homo sapiens*). From the margin of the macula lutea.

Fig. 430.—Entire cone visual cell of a mammal, man (*Homo sapiens*). From the fovea centralis. The progressive lengthening and narrowing from periphery to fovea culminates in this type.

Fig. 431.—Developing rod and cone visual cells of the chick one day after hatching. mi. mitochondria; o.g. oil globule; l.b. lentiform body.

Fig. 432.—Cone of man showing the ready degeneration of the outer segment into platelets which tend to be retained by an enveloping sheath.

PLATE II

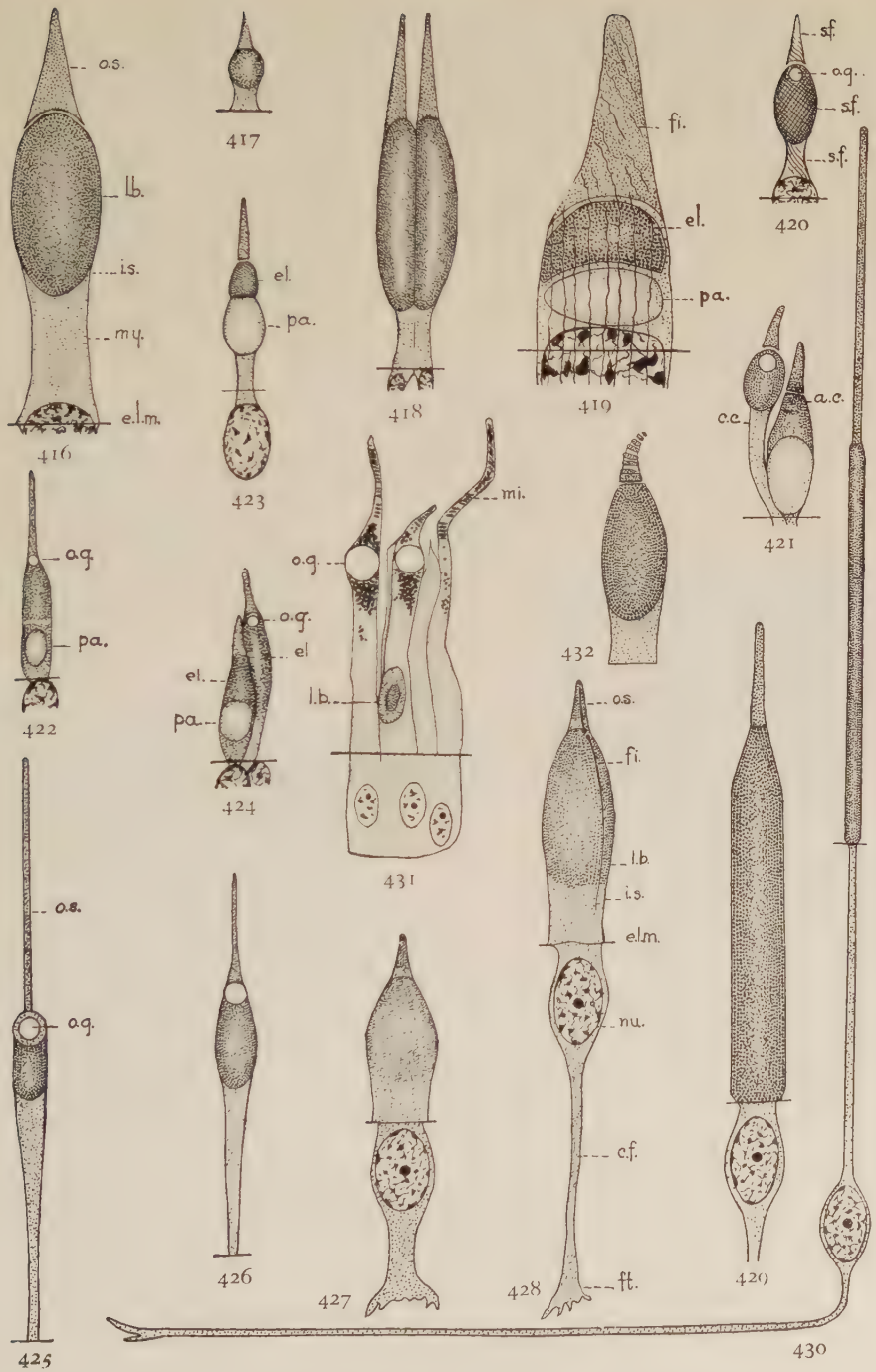

o.s.
l.b.
i.s.
m.y.
e.l.m.
416
417
e.l.
pa.
423
418
fi.
e.l.
pa.
419
sf.
o.g.
sf.
sf.
420
a.c.
c.c.
421
o.g.
pa.
422
mi.
o.g.
o.g.
el.
el.
pa.
l.b.
424
431
432
o.s.
o.g.
425
426
427
o.s.
fi.
l.b.
i.s.
e.l.m.
nu.
c.f.
ft.
428
429
430

PLATE III

Pigmented epithelium of representative vertebrates. The magnifications are uniform in all figures of the same series and are stated in each instance. The figures are in part original and in part adapted or combined from various monographs.

FIG. 433. Horizontal section of the pigmented epithelium of man, cut at the level of the nucleus and cell body. The shape of the cells and the arrangement of the mosaic are typical. × 800.

FIG. 434. Single pigmented cell of man in vertical section. From the fundus, outside the macula lutea. The three zones and their components are depicted. ba, cell base; bo. cell body; pr. cell processes; nu. nucleus; m.g. myeloid granules; lg. lipochrin globules; a.p. amorphous pigment granules; c.p. crystalline pigment needles. Myeloidin and lipochrin are said to be lacking in man but have been added to complete the composite picture of a typical pigment cell. × 1000.

FIG. 435. Vertical section of the pigmented epithelium of man, stained to show the terminal bar system (t.b.) as described by Verhoeff. × 300.

FIG. 436.—Crystalline pigment granules of a fish, the roach (*Leuciscus rutilis*). × 1000.

FIG. 437.—Crystalline pigment granules of a fish, the pike (*Esox lucius*). × 1000.

FIG. 438. Crystalline pigment granules of an amphibian, the leopard frog (*Rana pipiens*). × 1000.

FIG. 439. Crystalline pigment granules of a reptile, the lizard *Lacerta sp.*. × 1000.

FIG. 440.—Crystalline pigment granules of a bird, the sparrow (*Passer domesticus*). × 1000.

FIG. 441. Crystalline pigment granules of a bird, the pigeon (*Columbia livia*). × 1000.

FIG. 442.—Crystalline pigment granules of a mammal, the brown rat (*Mus norvegicus*). × 1000.

FIG. 443.—Crystalline pigment granules of a mammal, man (*Homo sapiens*). × 1000.

FIG. 444.—Vertical section of the retina of the alligator (*Alligator mississippiensis*) at a region where the tapetum is best developed. The guanin (fine stipple) occupies a broad zone with a narrow border of melanin (coarse needles) at the vitreal edge. × 665.

FIG. 445.—Vertical section of the retina of the alligator (*Alligator mississippiensis*) toward the margin of the tapetum. The zone of melanin increases at the expense of the narrowed guanin layer. × 665.

FIG. 446.—Vertical section of the non-tapetal area of the retina of the alligator (*Alligator mississippiensis*). Guanin is entirely absent. × 665.

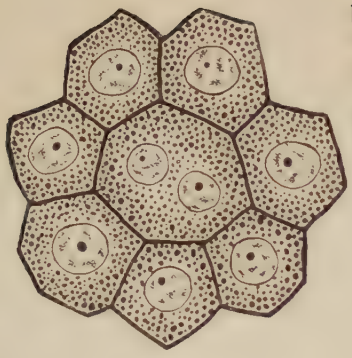
FIG. 447.—Vertica lsection of the retina of a diurnal fish, the perch (*Perca fluviatilis*). The pigment and visual cell zones are narrow. The pigment processes are coarse and never retract maximally but expand in a dense zone about the cones toward the external limiting membrane. × 230.

FIG. 448.—Vertical section of the retina of a nocturnal fish, the eel pout (*Lota vulgaris*). The pigment and visual cell zones are broad, to accommodate the fine, abundant rods situated at different levels as is characteristic of efficient dim-light vision. The pigment processes are slender and retract maximally but never expand beyond a broad, diffuse zone. × 230.

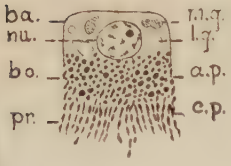
FIG. 449.—Vertical section of the retina of a fish, the horned pout (*Ameiurus nebulosus*). The retinal elements are represented in the positions characteristic of dim-light vision. The pigment is withdrawn toward the chorioid, thereby allowing maximal utilization of dim light. The visual elements assume similarly advantageous positions; the relatively insensitive cones are extended out of the way and the functioning rods are drawn down near the image plane. ch. chorioid; p.c. pigmented epithelial cell; p.g. pseudo-guanin; c.m. cone myoid; r.m. rod myoid; e.n.l. external nuclear layer. × 450.

FIG. 450.—For caption see second page following.

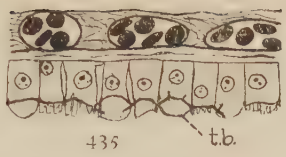
PLATE III



433



434



435

436 437 438 439 440

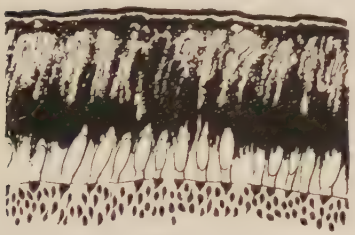
444

445

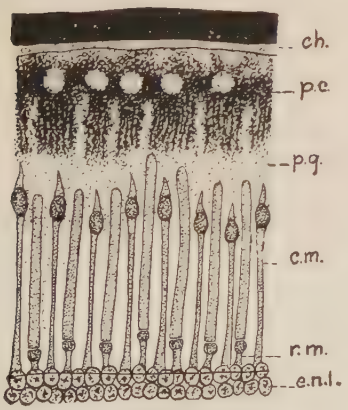
446

441 442 443

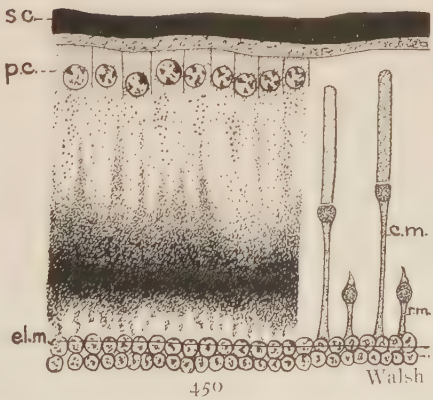
448



447



449



450

Walsh

PLATE III (cont'd)

FIG. 450.—Vertical section of the retina of a fish, the horned pout (*Ameriurus nebulosus*). The retinal elements are represented in the positions characteristic of bright-light vision. The pigment moves forward toward the external limiting membrane, thereby masking and isolating the visual rods and cones. At the right of the figure the mutually advantageous positions of the visual elements are indicated; the sensitive rods are elongated and so protected and out of use, the cones are shortened to the level of the image plane. sc. sclera; p.c. pigmented epithelial cell; e.l.m. external limiting membrane; c.m. cone myoid; r.m. rod myoid. × 450.

Section XXVI

CYTOLOGY OF THE INTERNAL EAR

CONTENTS

Section XXVI

PAGE

I. Osseous Capsule of Labyrinth 929
II. Perilymphatic Spaces 932
III. Membranous Labyrinth 934
Ductus Cochlearis—Cells of Claudius—Cells of Boettcher—Hair cells of papilla spiralis.
IV. Bibliography 959

Section XXVI

CYTOLOGY OF THE INTERNAL EAR

GEORGE E. SHAMBAUGH

STUDY of the cytology of the labyrinth centers about the three types of special sense organs formed in the membranous labyrinth: the organ of Corti in the cochlea, the macula of the utricle and saccule and the crista ampullaris of the semicircular canals. The examination of these structures presents problems of peculiar difficulty. In the first place, their extreme delicacy is such that to secure material satisfactory for histological study, that is, before the rapidly developing post-mortem changes have set in, requires an immediate fixation, not easy to accomplish because of the hard, impervious osseous capsule. In the second place, before histological sections can be made, this osseous capsule must be decalcified and the imbedding must be carried out with great care. Our knowledge of the cytology of these structures has been the result of much patience and perseverance. It is not surprising, therefore, that relatively few are the names of the investigators who have actually contributed to our knowledge of the internal ear.

I. OSSEOUS CAPSULE OF THE LABYRINTH

Before we undertake to describe the cytology of the membranous labyrinth it seems desirable to give a brief description of the osseous labyrinth in which the membranous structures are suspended. The osseous internal ear consists of a series of communicating cavities filled with perilymph encased in the petrous bone in which there hangs suspended a membranous labyrinth also made up of a series of intercommunicating cavities lined with epithelium and which form a closed system filled with a fluid called endolymph.

The osseous labyrinth is made up of a central chamber (Fig. 451), the vestibule, an elongated tube, the cochlea leading off anteriorly, and three semicircular canals from the posterior wall which communicate with the vestibule through five openings. The cochlear tube is coiled on itself in the manner of a snail shell and in man forms two and one-half coils.

The osseous capsule is made up of three layers (Fig. 452), a thin inner layer of ivory-like density formed from the endosteum lining the osseous cavities and quite free from blood vessels, receiving its nutriment through lymph spaces from the blood supplying the endosteum; an outer layer of less dense bone derived from the periosteum lining the posterior and middle brain fossae, and the inner wall of the tympanic cavity. This layer is penetrated by blood vessels from the surrounding structures. A third or middle layer is derived from cartilage which in the embryo forms the capsule of the labyrinth. This bone is denser where it lies next to the endosteal layer and becomes somewhat less dense where it borders the periosteal bone from which it receives its nutriment. The middle layer is peculiar in that it retains throughout life nests of embryonal cartilage. It is in this layer where those changes begin which transform the dense bone of the

capsule into a loose, spongy bone in those cases of progressive deafness known as otosclerosis.

The blood supply of the endosteal lining of the cavities of the osseous labyrinth comes from the labyrinthine artery which enters through the internal meatus. As a rule, no branches from this system enter the capsule and there exists, therefore, no communication between the blood vessels of the membranous labyrinth and those of the

FIG. 451.—Wood's metal cast of the osseous cavities of the human labyrinth, left ear: a, cupola cochleae; b, middle coil; c, fenestra cochleae; d, fenestra vestibuli; e, ampulla ossea posterior; f, crus commune; g, ampulla ossea superior; h, ampulla ossea lateralis; i, recessus ellipticus; j, recessus sphericus; k, basal coil.

osseous capsule. I was able to demonstrate that in the calf this does not hold and that at a number of places branches from the labyrinthine artery do enter the capsule. Not infrequently in the calf a large branch from the vessels of the dura penetrates the capsule to form the artery crus communi.

The osseous capsule is perforated by several openings. Conspicuous is the oval opening in the lateral wall of the vestibule, the *fenestra vestibuli*, which leads to the tympanic cavity and is closed by the footplate of the stapes. Immediately below this, separated only by a couple of millimeters, is a round opening, the *fenestra tympani*,

connecting that part of the vestibular end of the cochlea known as scala tympani with the tympanic cavity. This is closed by a strong membrane known as the membrana tympani secondaria (Fig. 455). Perforating the posterior bony wall of the vestibule is a channel, the *aqueductus vestibuli*, which opens on the posterior aspect of the petrous bone and transmits a tubular elongation, the ductus endolymphaticus, from the membranous labyrinth. This duct ends blindly in a small pouch, the saccus endolymphaticus,

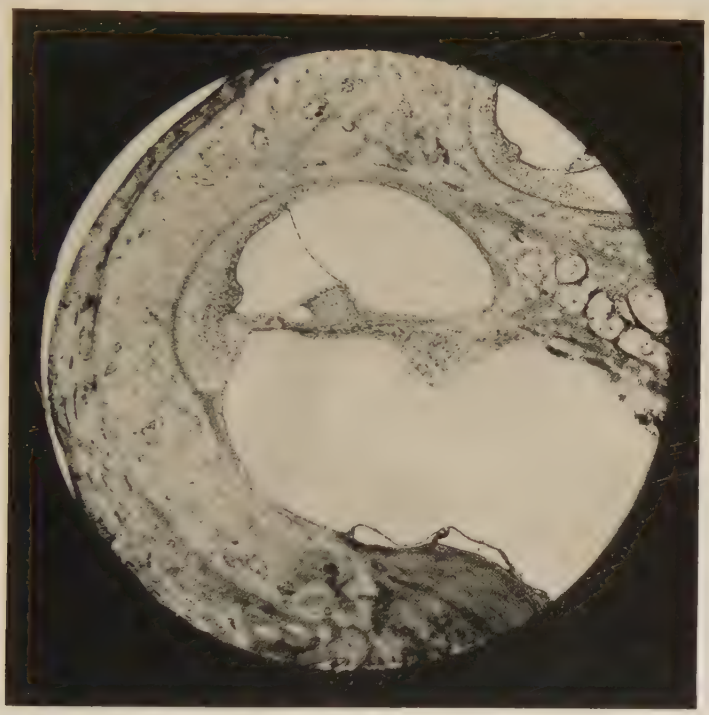


FIG. 452.—Section through basal coil of cochlea, pig. Note the demarcation between the three layers of the osseous capsule, layer of endosteal bone, layer of periosteal bone, and the substantial middle layer derived from the embryonal cartilaginous capsule. Note the large scala tympani and the scala vestibuli, the lamina spiralis ossea terminated by the crista spiralis, the bulbous enlargement beneath the lamina for encasing the spiral ganglion. Note the ligamentum spiralae Reissner's membrane and the structures of the membranous cochlea, the stria vascularis, the prominentia spiralis, the sulcus spiralis externis of the outer wall and the papilla spiralis resting on the membrana basilaris.

placed between the layers of the dura of the posterior brain fossa. In man and in many mammals the aqueductus vestibuli transmits a vein from the vessels of the internal ear.

Leading from the under-surface of the cochlear tube close to the point where this tube comes off from the vestibule is a narrow channel perforating the bony capsule and forming a passage between the scala tympani of the cochlea and the posterior brain fossa. This channel is known as the *aqueductus cochleae* and permits the perilymph of the labyrinth to join the fluid in the subarachnoid space. Along with this aqueductus cochleae is another small channel, *canalis cotunii*, for the passage of the main vein leading

from the labyrinth. In some of the lower animals, for example, the pig and the sheep, this vein of the aqueductus cochleae constitutes the sole venous drainage from the internal ear.

The only remaining openings in the osseous capsule are those found in the fundus of the internal meatus for the transmission of the cochlear and vestibular branches of the eighth nerve to the internal ear and for the passage of branches of the labyrinthine artery. Some have described a vein in the internal meatus which drains blood from the labyrinth. This vein I have not been able to verify.

The cone-shaped structure about which the cochlear tube is coiled is known as the *modiolus* (Fig. 454). It is made up of an osseous, connective tissue structure perforated by numerous channels for the transmission of blood vessels and branches of the cochlear nerve. Extending radially from the modiolus is a bony shelf partly separating the cochlear tube into two spiral chambers, the vestibular chamber above and the tympanic chamber below. This *lamina spiralis ossea* follows the spiral cochlear tube from the vestibule to the apex. The lamina is narrower at the apex of the cochlea and becomes gradually broader toward the base. It is perforated for the passage of nerves and blood vessels.

II. THE PERILYMPHATIC SPACES

A good way of acquiring a clear conception of the perilymphatic system of the labyrinth is by examining embryological preparations showing the development of these spaces. Such a series of sections I have prepared from the embryo of the pig. From these sections one sees the formation of the membranous labyrinth preceding the development of any perilymphatic spaces. In the cochlea the membranous structure consists of an oval, spiral tube, the ductus cochlearis. In the vestibule, one recognizes cross sections of the saccule and utricle, and posteriorly, the membranous semicircular canals. In a pig embryo measuring three and one-half centimeters long, no perilymphatic spaces are to be found, the membranous labyrinth being encased in a capsule of cartilage and embryonal connective tissue. The perilymphatic spaces are formed later by the transformation of cartilage into connective tissue and the absorption of the latter, leaving spaces which become filled with perilymph (Fig. 453). The formation of perilymphatic spaces about the membranous labyrinth remains, even in the adult, incomplete, so that the latter continues to be attached to the capsule at certain places. In the utricle and saccule the areas of attachment correspond to the maculae. In the semicircular canals the membranous tube remains attached along the outer convex surface, at the ampulla, particularly at the base of the crista (Fig. 469). In the cochlea one can readily trace the development of the perilymphatic spaces. These begin in the basal coil as two open spaces, one above, the other below the ductus cochlearis, forming respectively the scala vestibuli and the scala tympani. Separating the scala vestibuli from the membranous cochlea is the vestibular membrane, the membrane of Reissner, and separating the scala tympani from the membranous cochlea is the basilar membrane. While the ductus cochlearis becomes surrounded in this manner both above and below by peri-

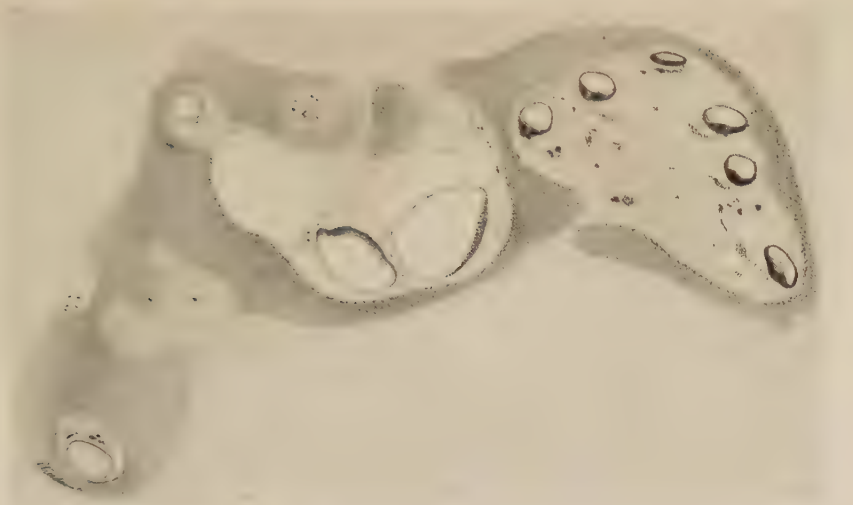


FIG. 453.—Pig embryo, 8 cm. long. Section passes through cochlea vestibule and horizontal and posterior semi-circular canals. Note the perilymphatic spaces developing in the vestibule and in the beginning of the basal coil. The cartilage about the semicircular canals has begun to be transformed into connective tissue which is later absorbed forming the perilymphatic spaces.

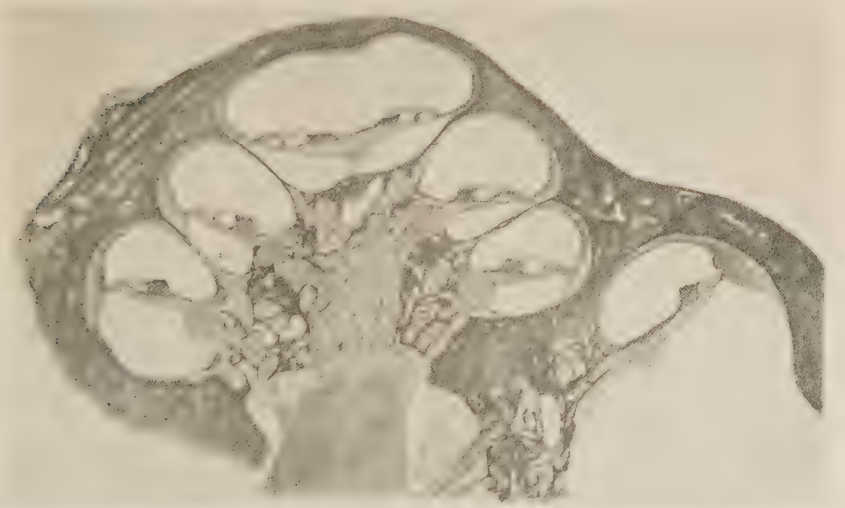


FIG. 454.—Section through cochlea of the domestic pig, adult, showing modiolus and cross section of the several coils of the cochlea.

lymph spaces, it remains permanently anchored to the surrounding osseous capsule along its outer wall (Fig. 454) by means of the intervening ligamentum spiralae and mesially to the osseous lamina spiralis by means of a conspicuous thickening, the crista spiralis, composed of connective tissue and epithelium, which rests on the outer rim of this osseous plate. The two perilymphatic spaces of the cochlea, the scala vestibuli above and the scala tympani below the ductus cochlearis, follow the spiral coils of the cochlea to its apex where they unite by means of a large opening, the helicotrema.

The walls of the perilymphatic spaces are lined by a thin layer of endothelium which in the region of the special sense organs becomes much thickened, has a rich capillary network of blood vessels and is perforated for the passage of terminal filaments of the eighth nerve. The endosteum lining the perilymphatic spaces carries the arteries and veins to and from the capillary system under the areas of modified epithelium which form the special sense organs. Except for these areas, the endosteum is as a rule quite free from any capillary vessels.

III. THE MEMBRANOUS LABYRINTH

The term membranous labyrinth is applied to the epithelial structures which form a series of cavities constituting a closed system filled with endolymph and which hang suspended in the perilymph of the osseous labyrinth. The several chambers of the membranous labyrinth communicating with each other are as follows: the ductus cochlearis forming the membranous cochlea unites with the saccule by means of the ductus reuniens. The saccule unites with the utricle by means of a small tube from each which unite to form the ductus endolymphaticus. The latter passes out through the aqueductus vestibuli ending in the saccus endolymphaticus. The three membranous semicircular canals communicate with the utricle through five openings, two of the canals uniting to form a single tube, the crus communi.

The Ductus Cochlearis. The membranous cochlea in the adult forms a triangular-shaped tube, the ductus cochlearis, which, beginning in the vestibule, follows the spirals of the cochlea to its apex. It contains several areas of highly specialized epithelium. The details of the structures are repeated with but slight variations from one end of the cochlea to the other (Fig. 474), so that a description of them as seen in a cross section of one of the cochlear coils suffices very well for the remainder of the cochlea.

The ductus cochlearis is attached to the outer wall of the osseous cochlea by means of the ligamentum spiralae and mesially to the lamina spiralis ossea by means of the crista spiralis (Fig. 452). It is separated from the scala vestibuli above by the membrana vestibularis, Reissner's membrane, and from the scala tympani below by the basilar membrane stretched between the crista spiralis and the spiral ligament.

The *ligamentum spiralae* is a thickening of the endosteal layer of connective tissue lining the osseous cochlea. It forms the outer wall of the vestibular space above and of the tympanic space below. It is thickest along the middle area where it forms a conspicuous v-shaped spiral ridge for the attachment of the basilar membrane. Both above and below this point it tapers, gradually becoming thinner and thinner until it passes into the undifferentiated, flat layer of endothelium lining the osseous tube. It is thickest in the basal coil, becoming very much thinner toward the apex of

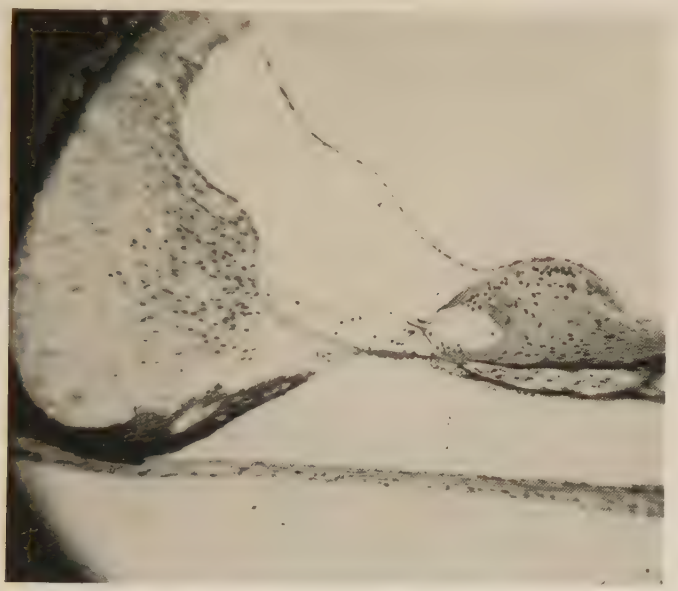


FIG. 455.—Photomicrograph. Section of cochlea at beginning of basal coil. Section cut through round window. Note membrana tympani secondaria below scala tympani, the shape of the spiral ligament. In the papilla spiralis note the tunnel of corti, space of Nuel and outer tunnel.

the cochlea. Where it is attached to the endosteum it forms a loose, areolar structure. Where it forms the v-shaped ridge for the attachment of the basilar membrane it is composed of a dense substance almost free from cell nuclei and presenting the appearance of elastic tissue. When viewed in radially-cut cross sections in the middle of the basal coil, its appearance suggests a structure intended for producing tension on the basilar membrane. Viewed in sections made toward the apex of the cochlea or near the beginning of the basal coil such a rôle does not seem possible (Figs. 454 and 455).

The *crista spiralis* is an elevation of connective tissue and epithelium resting on the outer free margin of the osseous spiral lamina. Its size varies in different parts of the cochlea as well as in different species. Peripherally the crista terminates in two lips, the *vestibular lip* above, to which the vestibular membrane and the membrana tectoria are attached, and the *tympanic lip* below, to which the basilar membrane is attached (Fig. 456). Between the two is a small depression called the *sulcus spiralis internis*. A single layer of large, clear, cuboidal cells lines this sulcus. The upper margin of the vestibular lip as seen in cross section appears smooth but viewed from above is seen to be indented by a series of depressions separated by ridges which become more conspicuous toward the free margin of the lip and constitute the *teeth of Huska*.

The *tympanic lip* of the crista spiralis projects peripherally somewhat beyond the vestibular lip. It forms a direct extension of the osseous spiral lamina and is made up of two plates. The vestibular plate forms the floor of the sulcus internis and is lined by a layer of clear, cuboidal cells. The structure of this plate is clear and quite free from nuclei except in its deeper layer immediately covering the fibers of the cochlear nerve. The tympanic plate consists of connective tissue cells covered by a thin layer of endothelium. This plate is perforated by numerous channels for the passage of nerve fibers and blood vessels.

Attached to the mesial elevation of the vestibular surface of the crista spiralis is a thin membrane which stretches diagonally across the cochlear tube to near the upper margin of the spiral ligament and separates completely the ductus cochlearis from the scala vestibuli. This is the *membrana vestibularis* or Reissner's membrane (Figs. 452 and 455). It consists of two extremely thin layers of cells, the nuclei from which form minute nodular elevations scattered sparsely along the membrane. The outer layer is made up of connective tissue cells and the inner layer of epithelium. The membrane in most mammals including man is quite free from blood vessels. In the sheep, the calf and the rabbit I have found in the membrane of Reissner a network of blood vessels arising from those which radiate out along the lamina spiralis and anastomosing with those of the spiral ligament.

The *membrana basilaris* (Fig. 456) is a direct continuation of the tympanic lip of the crista spiralis. It spans the space intervening between this and the spiral ligament. Resting partly on the tympanic lip of the crista but more on the basilar membrane is a highly specialized sense organ, the papilla spiralis, Corti's organ. The inner part of the basilar membrane known as the *zona tecta* extends peripherally to the outer end of the footplate of the outer pillar of Corti. The outer part, known as the *zona pectinata*, extends from this point to its attachment to the spiral ligament.

The basilar membrane proper is composed of thin fibers, the direct extension of those forming the tympanic lip of the crista spiralis. They

extend from this point radially across to the spiral ligament. In the zona tecta these fibers are very thin and quite free from cell nuclei, whereas in

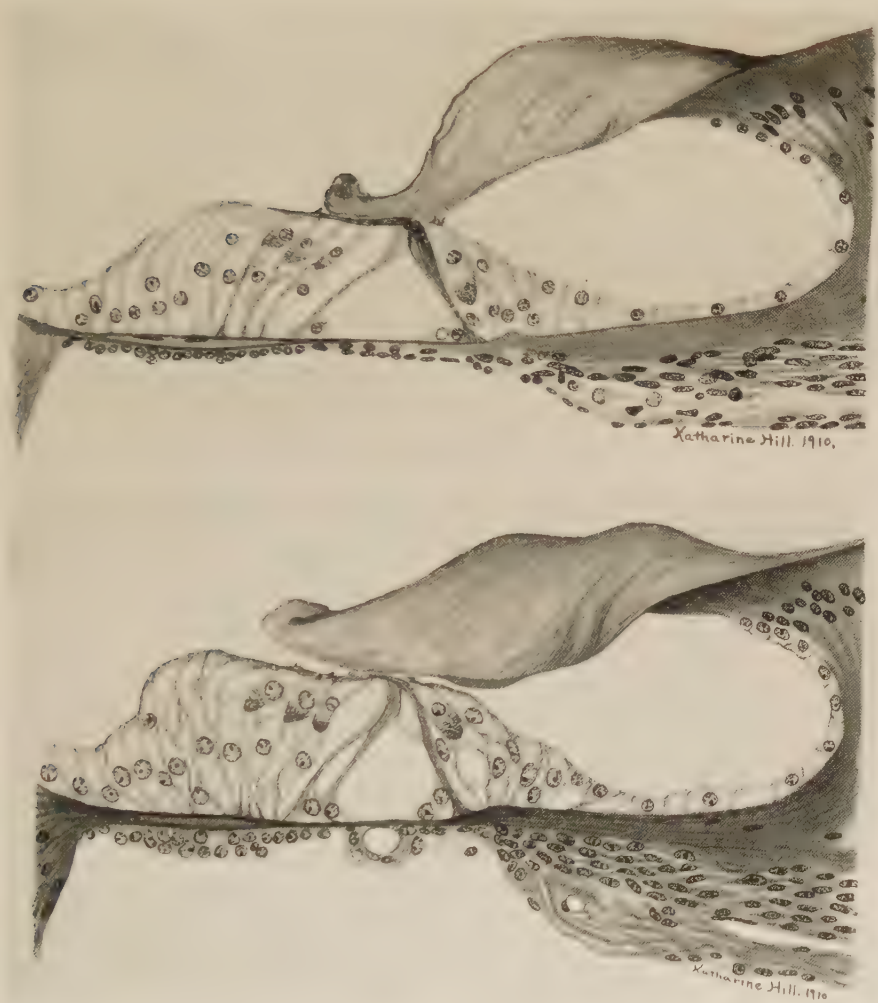


FIG. 456.—Papilla spiralis, Corti's organ, in middle of basal coil, pig.
FIG. 457.—Papilla spiralis, Corti's organ, basal coil, showing blood vessel under tunnel, pig.

the zona pectinata cell nuclei are usually seen. Along the lower side of the membrane is a layer of connective tissue cells, the remains of tissue which in the embryo occupies the area of the scala tympani. This layer varies in

thickness in different species as well as in different parts of the same cochlea. Blood vessels are attached to the under-surface of the basilar membrane. These vessels arise from those which radiate from the modiolus out along the lamina spiralis. The terminal capillary loops form one and at times two spiral vessels attached to the under-surface of the zona tecta directly under the tunnel of Corti (Fig. 457). The zona pectinata remains as a rule quite free from blood vessels. In the domestic pig, however, I have been able to find throughout the basal and second coils of the cochlea an occasional vessel radiating from the spiral vessel under the tunnel of Corti out along the zona pectinata to anastomose with the blood vessels of the spiral ligament. The space separating the crista spiralis from the spiral ligament is broadest at the apex of the cochlea becoming gradually narrower toward the proximal end of the basal coil. The membrana basilaris which spans this space is, therefore, broader toward the apex and becomes narrower toward the base of the cochlea.

The membrana basilaris is usually regarded as that structure in the cochlea which by responding to the physical impulse of sound waves brings about the interaction between the hairs of the hair cells of Corti's organ and the membrana tectoria which result in the stimulation of the terminal filaments of the cochlear nerve and produce the sensation of hearing. By many this response is supposed to be the result of physical resonance, each tone of the scale producing vibrations in different areas of the basilar membrane and thus stimulating different groups of hair cells for each tone. These speculations regarding the function of the basilar membrane have hardly considered sufficiently all the anatomical factors relating to this structure. For example, the thick layer of connective tissue cells attached to the under-surface often causes this membrane to lose all resemblance to a vibrating structure. Toward its lower end the basilar membrane becomes a thick, v-shaped structure (Fig. 463) quite incapable of acting as a vibrating mechanism and finally in the area most essential for vibrating, that is, in the zona tecta, the membrane is a vascular structure, which would seem to destroy the possibility of its responding in the same manner at all times to the same impulses, an absolute requirement for a structure which fills the rôle attributed to this membrane. It would seem, moreover, from analogy with the other end organs in the labyrinth, that the stimulation of the hair cells of Corti's organ should result from movements in the superimposed structure, the membrana tectoria, since the stimulation of the hair cells in both the crista and macula is the result of movements in their analogous structures, the cupola and otolith membrane respectively.

The outer wall of the ductus cochlearis rests on the vestibular half of the spiral ligament, the part lying between the attachment of the basilar membrane below and the membrane of Reissner above. This wall is formed into two unequal, concave segments separated by an elevation called the

prominentia spiralis (Fig. 452). The larger segment lies above the prominence and is occupied by a structure rich in blood vessels called the stria vascularis. The smaller segment lies below, that is, between the spiral prominence and the attachment of the basilar membrane and is known as the *sulcus spiralis externis*. The epithelium in these two areas presents interesting peculiarities.

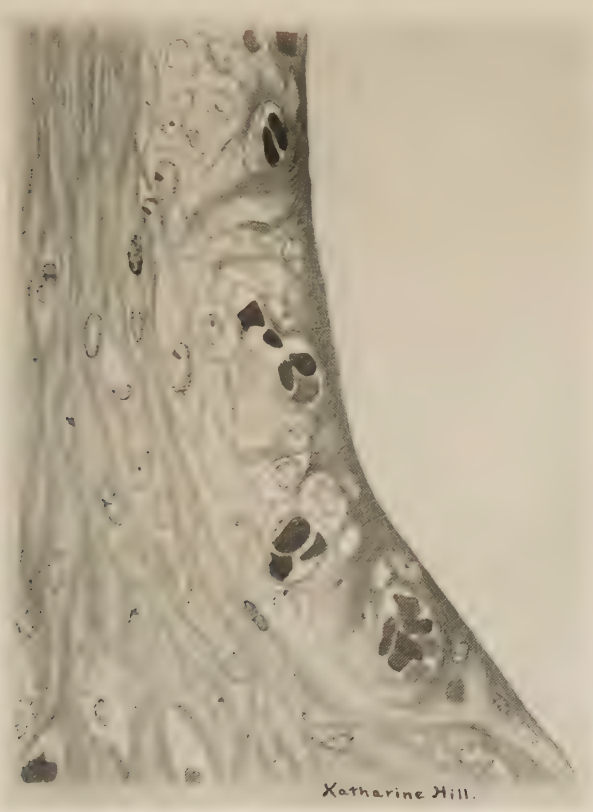


FIG. 458.—Stria vascularis, adult pig.

The *stria vascularis* extends from the spiral prominence below to the attachment of the membrane of Reissner above. Occasionally a small space covered by a single layer of undifferentiated, flat epithelial cells separates the upper end of the stria from the attachment of Reissner's membrane. The stria takes its name from a peculiar rich supply of blood vessels which form a system of capillary loops quite distinct from those of the ligamentum spiralae. These capillary loops follow for the most part the direction of the coils of the cochlea and appear in cross section as spiral vessels. The arterial

supply comes from an occasional small vessel given off from the arteries which radiate out from the modiolus and arch over the scala vestibuli lying in its endosteum. The venous blood is likewise drained by an occasional small vessel which, leaving the stria, joins with the veins of the spiral ligament which run back to the modiolus lying in the endosteum of the scala tympani. The blood vessels of the stria (Fig. 458) lie for the most part directly under the surface layer of epithelium which lines the ductus cochlearis. This surface layer of epithelium is made up of a single layer of cells taking a deeper stain and having long, fibrillar protoplasmic processes which penetrate the deeper layers of the stria extending to the cells lying next to the spiral ligament. Beneath this surface layer of peculiarly modified epithelium is a deeper layer of the stria made up of cells the origin of which has long been in dispute. By some these cells have been regarded as having an epithelial origin, that is, derived from the single layer of epithelium which in the embryo occupies this area. By others the cells of the deeper layer have been recognized as connective tissue cells derived from the cells of the underlying spiral ligament. Those who hold the former view recognize in the stria vascularis a truly vascular epithelium. I have been able, by studying the development of the stria, to demonstrate that the cells of the deeper layer are in part derived from the surface layer of embryonic epithelium and in part come from the connective tissue cells of the spiral ligament. This demonstration was accomplished as follows: I found that in the embryo pig measuring from three to eight centimeters, a single layer of epithelium lining this area was separated from the underlying cells of the spiral ligament by a distinct basement membrane which, when using the Mallory stain, takes a deep blue, sharply differentiating it from the surrounding structures. This blue-staining basement membrane persists until several layers of cells are included between this membrane and the free surface. These can only be epithelial cells. At a later stage when blood vessels have begun to penetrate to the surface layer of epithelium, shreds of this blue-staining membrane are still found occupying a place midway between the cells forming the deeper layer. The cells between this membrane and the spiral ligament seem, therefore, clearly to be derived from the latter structure and are, therefore, of connective tissue origin.

It would seem from its rich blood supply that the stria has an important physiological rôle. By some it has been regarded as a secretory mechanism for supplying the endolymph. Since a true secretory mechanism, a glandular structure, has been discovered by me in the epithelium of the sulcus externis, to be described below, there would seem to be no need for attributing such a function to the stria, and one wonders whether this vascular structure may not be a mechanism for regulating the endolymph pressure or whether it may not constitute a mechanism for providing those pulsations to the endolymph which seem to be necessary to produce the continu-

ous flow of tonus impulses to the skeletal muscles which clearly emanate from the cristae.

The epithelium covering the *prominentia spiralis* (Figs. 459, 461 and 462), is made up of a single layer of cuboidal cells directly continuous with the surface epithelium of the stria and having the same deeply staining property as these cells. The outlines of this layer of cells are sharply defined

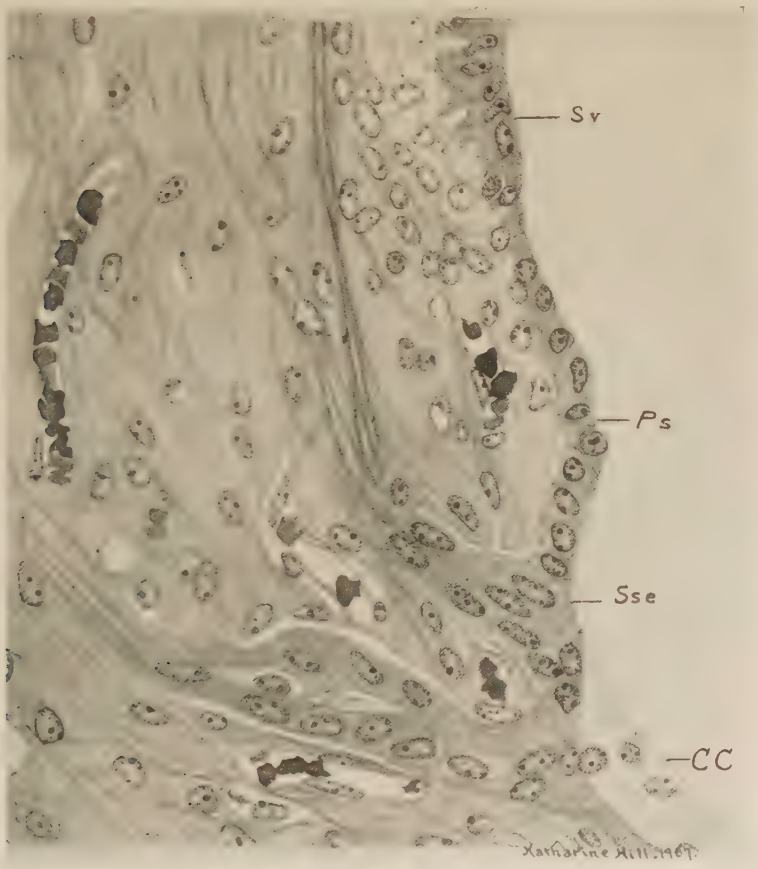


FIG. 459.—Section of outer wall of ductus cochlearis showing from above downwards; Sv, stria vascularis; Ps, prominentia spiralis; Sse, sulcus spiralis externis and cc, Cells of Claudius.

and they have none of the fibrillar, protoplasmic processes peculiar to the surface epithelium of the stria.

The cells lining the *sulcus spiralis externis* are the direct continuation of those covering the spiral prominence and like the latter these cells take a deep stain. They resemble the surface epithelium of the stria in the possession in some areas of long, fibrillar protoplasmic processes (Figs. 459 to

461). In the cells of the sulcus externis these processes are much longer than are those of the stria. They penetrate deeply into the spiral ligament itself. In the basal coil of the cochlea of the pig and the guinea pig I found clumps of these cells extending deeply into the structure of the spiral ligament and with long, fibrillar processes which could be traced to the endosteum and to the level of the upper margin of the stria. Horizontal sections cut parallel with a coil of the cochlea show that between the areas where clumps of these cells actually penetrate the spiral ligament (Fig. 460), the single layer of surface epithelium sends out long fibrillar protoplasmic processes into the structure of the spiral ligament. Such fibrillar processes

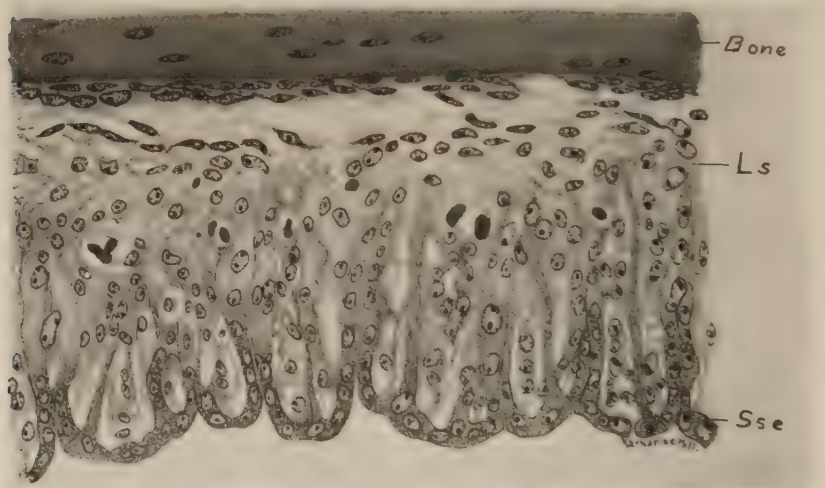


FIG. 460.—Horizontal section cut through the spiral ligament in the region of the sulcus spiralis externis. Sse, sulcus spiralis externis; Ls, ligamentum spiralis; B, bone.

have also been observed in man. In my investigation of the epithelium of the sulcus externis I found that occupying the center of these clumps of epithelial cells there is a small tubule which can be traced to the free surface of the sulcus externis. Occasionally the tubule widens into a cyst-like cavity surrounded by epithelium. Only occasionally was I able to discover similar fibrillar processes from this epithelium in the second coil of the cochlea and in the basal coil only did I observe the presence of tubules.

It would appear clear that we have here a secreting mechanism, possibly the mechanism for replenishing the endolymph. What the function may be of the fibrillar processes is not clear. Their construction suggests a contractile mechanism.

The Cells of Claudius. Directly continuous with the epithelium of the sulcus externis and not infrequently covering in part these cells is a single layer of tall, clear cells, which line the lower surface of the sulcus externis and extend along the basilar membrane until they reach the elevation of cells forming the outer support of the papilla spiralis, Corti's organ. These are the cells of Claudius (Figs. 459, 461 and 462). The number of these cells seen in a cross section differs widely in different parts of the cochlea and in the same part in different species.

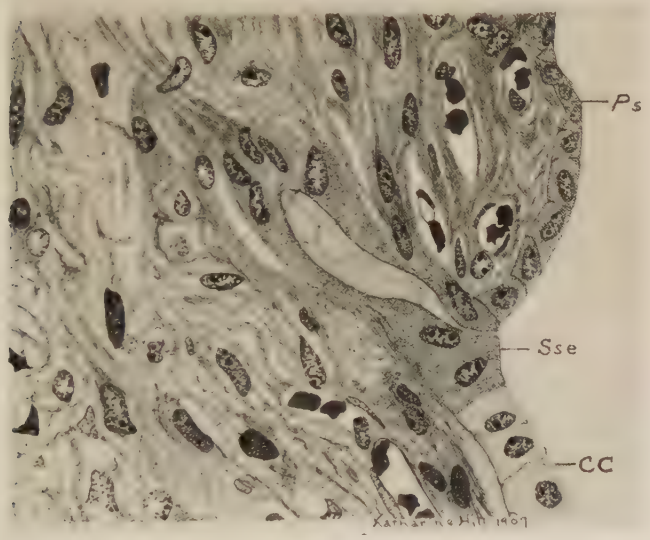


FIG. 461.—Section cut from the ligamentum spiralae showing epithelium covering of the Ps, prominentia spiralis; Sse, sulcus spiralis externis; cc, Cells of Claudius. Note penetration of the epithelium of the sulcus externis into the spiral ligament forming glandular structure.

The Cells of Boettcher. In certain areas in the basal coil, resting directly on the zona pectinata of the basilar membrane and covered over by the cells of Claudius, are found small clumps of cells, never more than three or four showing in a single section, differentiated sharply from the clear cells of Claudius by their finely granular protoplasm which takes a deep stain, the so-called cells of Boettcher (Fig. 463).

The *papilla spiralis*, Corti's organ, is made up of a highly specialized group of epithelial cells which rest in part on the tympanic lip of the crista spiralis and in part on the membrana basilaris, completely covering the

zona tecta and most of the zona pectinata. The papilla contains several spiral rows of peculiar hair-bearing cells and an elaborate mechanism of supporting cells by means of which the hair cells hang suspended in the endolymph anchored only by their top and base. The papilla in complexity of structure is comparable only to that of the retina.

The cells actually taking part in providing support for the hair cells are buttressed on either side by a mass of high columnar cells known as *border*

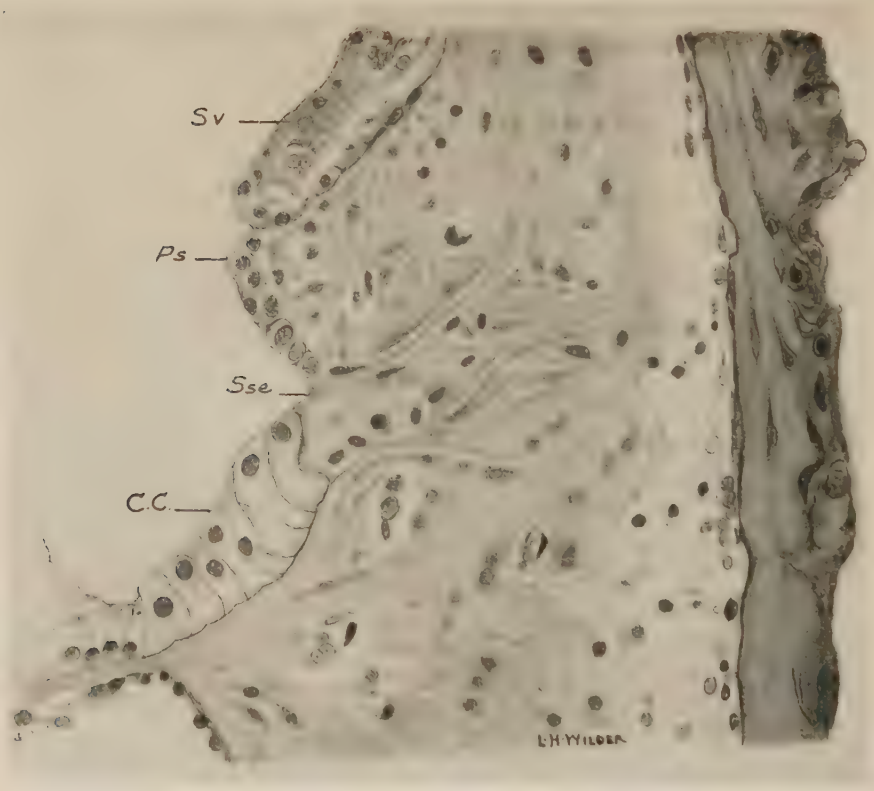


FIG. 462.—Section through the vestibular segment of the spiral ligament showing Sv, stria vascularis; Ps, promentia spiralis; Sse, sulcus spiralis externis; cc, Cells of Claudius.

cells. Those on the outer side of the papilla rest on the zona pectinata of the basilar membrane and are called the *cells of Hensen*. The shape and size of this mass of border cells differ widely in different species as well as in different parts of the same cochlea. They are banked closely against the

supporting cells of Deiters. On the mesial side of the papilla the inner row of hair cells with their underlying supporting cells are buttressed by a mass of tall, clear cells which rest on the labium tympanicum of the crista and reach to the level of the top of the hair cells. From this level the border cells gradually become shorter as they recede from the hair cells until they pass without any line of demarcation into the layer of flat, cuboidal cells lining the sulcus internis (Figs. 456 and 468).

The *supporting mechanism* for the hair cells of Corti's organ consists of two groups of highly specialized cells with an elaborate development of intercellular supporting fibers, the *pillar cells* and the *cells of Deiters* and an elaborate surface plate for encasing the heads of the hair cells, the *lamina reticularis*.

The *pillar cells* consist of two spiral rows, an inner and outer row, which joining above spread out below so as to encase a triangular space filled with endolymph, the *tunnel of Corti*. The inner pillars rest on the tympanic lip of the crista spiralis; the outer pillars on the zona tecta of the basilar membrane.

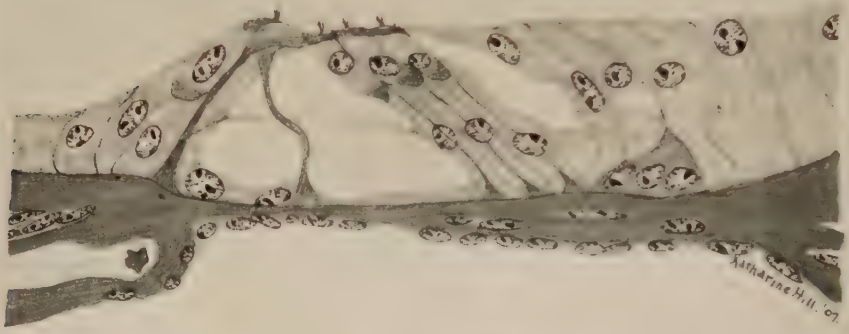
Near the proximal end of the basal coil the outer and inner pillars are of the same length and form with the basilar membrane an equilateral triangle. Throughout the remainder of the cochlea the inner pillars are shorter and placed more nearly at right angles to their supporting base than are the outer pillars. Each pillar cell has a broad, protoplasmic base which spreads out to form a complete covering for the floor of the tunnel space and contains cell nuclei. From the outer pillar cells a similar protoplasmic base extends across the floor of the space of Nuel to join with a similar process from the innermost row of Deiters' cells (Fig. 468). The outer pillars are separated from each other so that free communication exists between the endolymph in the tunnel of Corti and that in the space of Nuel which separates the outer pillars from the outer group of hair cells.

Each pillar encases a number of deeply staining parallel fibers which at the base spread out to encase a central cone. The fibers of the inner row of pillars spread out above into two sets, the one bending mesially to encase the heads of the inner row of hair cells, the other bending outward to encase the heads of the outer pillars extending to interlock with the elaborate surface plate of the papilla, the lamina reticularis.

The *cells of Deiters* are supporting cells for the outer group of hair cells, one for each cell. They form three and sometimes four rows of tall, faintly staining cells resting on the basilar membrane and placed between the space of Nuel mesially and the cells of Hensen peripherally. Each cell contains a large nucleus placed midway between the basilar membrane and a laterally placed facet for receiving the base of the hair cell which it supports. About

two-thirds of the space between the basilar membrane and the lamina reticularis is occupied by the cells of Deiters, the upper one-third being

463



464

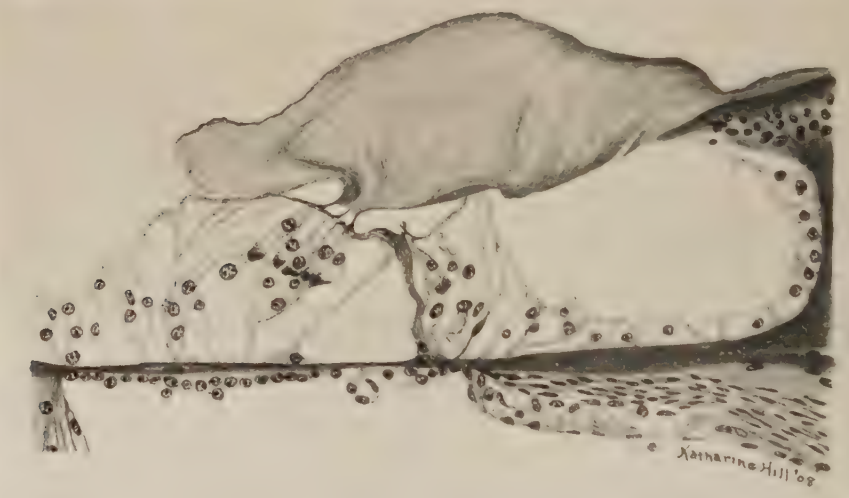


FIG. 463.—Section through papilla spiralis near proximal end of basal coil showing absence of vibrating basilar membrane, tunnel of Corti, space of Nuel and second tunnel lying between the cells of Hensen and the cells of Deiters; also three darkly staining cells of Boettcher lying under the outer border cells of the papilla.

FIG. 464.—Papilla spiralis showing attachment of hairs of the outer cells to membrana tectoria; also attachment of stripe of Hensen to the inner border cells which have been drawn out as the result of contraction of the tectorial membrane.

occupied by the hair cells. A conspicuous feature of the cells of Deiters is the presence of a bundle of deeply staining intercellular supporting fibers. Like the fibers of the pillar cells these spread out in a cone-shaped

base where the cells rest on the basilar membrane. Near the top of the cells these fibers divide into two groups. One group forms a veritable cup for receiving the base of the hair cell. The other group of fibers continues into an open space between the hair cells until it reaches the level of the surface plate of the papilla where it joins to a flat, stellate headpiece which enters into the formation of the lamina reticularis, the structure for supporting the heads of the hair cells. This group of fibers does not pass directly upwards to the surface of the plate but bends over to join the lamina, two or three cell rows distant. In the cross sections of the cochlear coil most useful for the study of Corti's organ these fibrillar processes from Deiters cells appear as a series of two or three dots lying in the open space between the hair cells.

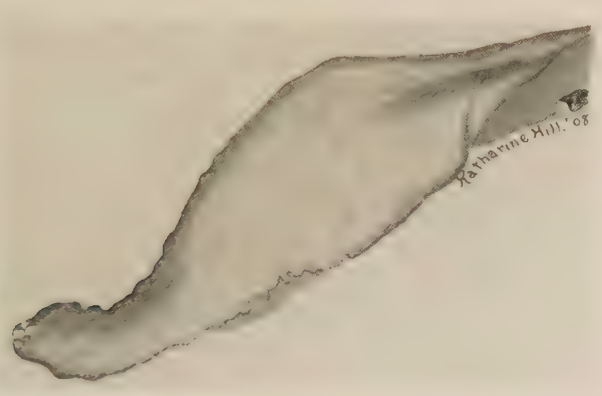


FIG. 465.—Membrana tectoria; the usual shape assumed after shrinking from fixation. Note stripe of Hensen along the under surface.

The *lamina reticularis* is an important part of the supporting mechanism for the outer group of hair cells. Mesially this plate is attached to the upward curving supporting fibers from the inner pillars of Corti and peripherally to fibers from the outer row of Deiters cells. Viewed from above it appears as a mosaic made up of an orderly arrangement of the headpieces from the prolongation of Deiters cells and a series of rings for receiving the heads of the hair cells.

The *space of Nuel* is an open space placed between the outer pillars of Corti and the inner row of the outer group of hair cells and Deiters cells. The endolymph in this space communicates freely with that in the tunnel of Corti by means of passages between the outer pillars. Near the proximal end of the basal coil is another space located peripherally to the outer row of hair cells and called the *second tunnel* (Figs. 455 and 463). It is sometimes described as placed mesially to the outermost row of Deiters' cells. My own preparations show it as lying between these cells and those of Hensen.

As anomalies in this supporting mechanism I have found occasionally three instead of two pillars of Corti (Fig. 466).

The Hair Cells of the Papilla Spiralis. The special sense structure of Corti's organ in which the transformation of the physical impulse of sound waves to a nerve impulse takes place is a peculiar hair-bearing cell of which there are two groups arranged in rows following the spirals of the cochlea, an inner and an outer group. The cells are unusually delicate and undergo post-mortem changes so quickly that it is difficult to secure preparations suitable for study.

The *inner group* of hair cells consists of a single row of cells wedged closely in between the inner pillar and the group of tall, inner border cells.

FIG. 466.—Anatomical variation, papilla spiralis near beginning of basal coil; three pillars of Corti.

They are somewhat larger than the hair cells of the outer group, being distinctly broader and somewhat longer than the latter (Fig. 468). They appear to be even more delicate than the outer hair cells or else they are so placed that fixing agents reach them less readily, for it is more difficult to secure preparations not showing more or less advanced post-mortem changes. The base of these cells is rounded where it rests on the supporting cells. The latter have none of the intercellular supporting fibers so conspicuous in the supporting cells for the outer group. The fact that this row of inner hair cells rests firmly against the inner pillar may render such a supporting mechanism unnecessary. The protoplasm of these cells, like that of the outer group, is finely granular and takes a stain but feebly. Toward the base of the cell these granules become larger and take a deeper stain. The appearance is not unlike sediment seen in the lower part of a glass flask. Something less than the lower one-third of the cell is occupied by these larger granules.

The *outer group* of hair cells is arranged in three and sometimes four spiral rows. The cells are cylindrical in form with the nuclei placed usually in the lower half. Rings of the lamina reticularis encase the head while the rounded base rests on a lateral facet from the underlying supporting cell of Deiters. A bundle of the intercellular supporting fibers from the latter forms a cone-shaped cup of longitudinally arranged, radiating fibers which encase the lower one-third of the hair cell. In the lower pole one finds the same sediment-like accumulation of granules taking a deep stain as seen in the inner group of hair cells. The terminal branches of the cochlear nerve can be traced to the base of the hair cells. The filaments for the outer group can be seen crossing the tunnel passing between the outer pillars and across the space of Nuel (Fig. 463).

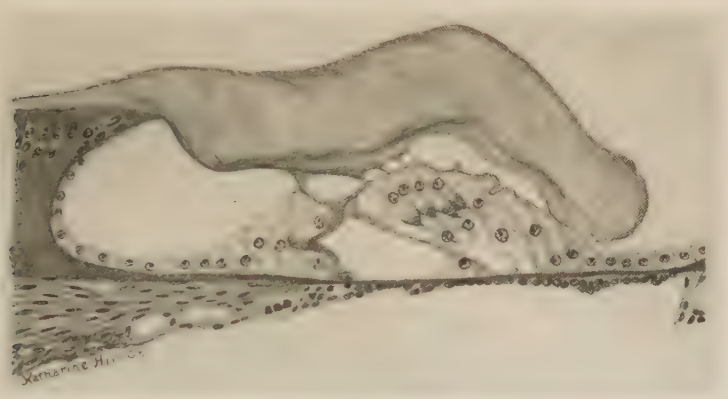


FIG. 467.—Papilla spiralis showing attachment of the hairs of the outer hair cells to membrana tectoria; also attachment of stripe of Hensen to inner border cells.

There is still to be considered one of the most important structures of Corti's organ, the *membrana tectoria* or *Corti's membrane*. It is generally accepted that the actual stimulation of the hair cells results from an interaction between their projecting hairs and this superimposed membrane. There persists a difference of opinion as to how this interaction is brought about; that is, whether it results from movements transmitted to the hair cells by the basilar membrane vibrating in response to the physical impulse of sound waves or whether the interaction is brought about by movements of the membrana tectoria itself. The close resemblance of the membrana tectoria to the cupola of the crista ampullaris makes it probable that the two structures stimulate their respective hair cells in much the same manner, that is, by themselves responding to impulses transmitted from the endolymph.

Because of its delicate structure the membrana tectoria is readily acted upon by fixing agents and undergoes in the process of preparation marked

distortions, particularly shrinking. For this reason it has proved to be an elusive structure for study. Nevertheless a number of facts regarding its make-up and the relation it holds to the cells of the papilla spiralis have been established. The origin of the membrane is not entirely clear, for, whereas in the embryo it appears to arise from the elevation of epithelium from which Corti's organ is formed, in the fully developed labyrinth the

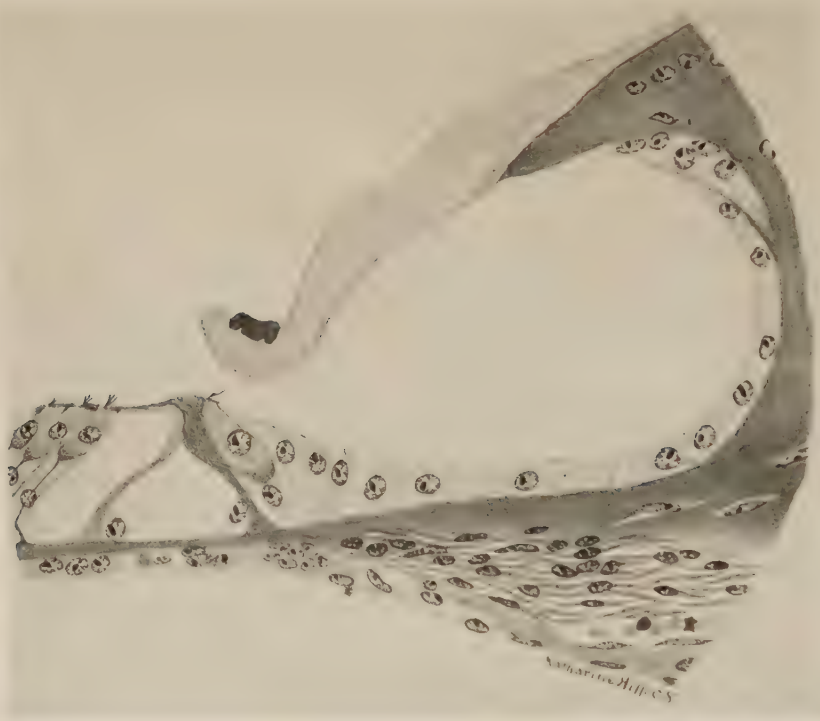


FIG. 468.—Papilla spiralis near beginning of basal coil; membrana tectoria shrunken away from the hair cells.

appearance suggests an outgrowth from the epithelium covering the labium vestibularis of the crista spiralis.

For convenience of description the membrane may be divided into two parts, that part which rests on the crista and the part which extends out over the papilla spiralis (Figs. 456, 457 and 465). The part which rests on the crista presents a fairly constant appearance, for this part is not altered by shrinking. Its beginning can be traced almost to the point where Reissner's membrane is attached. From this point it becomes gradually thicker toward the free edge of the vestibular lip. Beginning in this part striations

appear which radiate outwards and downwards to terminate at the lower border of the part of the membrane which projects out over Corti's organ extending to the very tip of the membrane. These striations have been

FIG. 469.—Cross section of semi-circular canal and ampulla showing relations of membranous canal and ampulla to the bony canal, crista ampullaris and cupola, pig.

described as fibrillae but from an examination of sections cut from a membrane that has become distorted and curled upon itself, one fails to recognize the cut ends of fibrillae, from which it would appear that the striations may represent a lamellar rather than a fibrillar structure.

In preparations with a minimum of distortion the part of the membrane which projects beyond the crista shows a structure that is broadest in the middle zone and which tapers gradually towards either end (Fig. 457). It is apparent that it projects well out beyond the outer hair cells, although in the proximal end of the basal coil it is usually lifted and pulled away from the papilla (Fig. 468). Even so it is often possible to find remaining shreds which stretch between the tip of the membrane and the cells of the papilla.

FIG. 470.—Ampulla from the horizontal semi-circular canal of pig. Note the prominent crista surmounted by the cupola; also larger area of hair cells on the canal side of the crista. Note the macula crista utriculae to the left of the crista and membranous ampulla filling the space of the osseous ampulla.

As a rule, the membrane, as the result of its shrinking, is found lifted quite free from Corti's organ and for a long time the true relation of these structures was not recognized. I was the first to demonstrate that the hairs of the hair cells of Corti's organ are normally in contact with and adherent to the under-surface of the membrana tectoria, a fact that has since been verified by a number of investigators (Figs. 464 and 467).

Along approximately the middle of the under-surface of that part of the membrane which projects beyond the crista is a more or less conspicuous spiral band or thickening quite free from striations. This is called the stripe of Hensen (Figs. 457, 464, 465 and 467). The function of this band has been made clear by my study of this structure. It constitutes a facet for anchoring the delicate membrana tectoria to the elevation of tall border cells which buttress the inner row of hair cells. Figures 464 and 467 show typical shrinking of the membrane, but the attachment of the hair cells of the outer group is still preserved, as is the anchorage along the stripe of Hensen. The whole of the membrane, that is, the vestibular as well as the tympanic aspect, is covered by a thin membrane with a delicate network of fibers. Because of the relations just described, whereby the tectorial membrane is seen to be attached to the papilla spiralis, an inner tunnel is formed, filled with endolymph, of which the sulcus spiralis internis forms the mesial wall and the papilla spiralis the outer boundary.

V. THE MEMBRANOUS STRUCTURES IN THE VESTIBULE AND SEMICIRCULAR CANALS

The *membranous structures in the vestibule*, the saccule and utricle, with their maculae and the membranous semicircular canals, each with its end organ, the crista ampullaris, constitute a mechanism that is concerned with the sensing of space and equilibration as distinguished from the acoustic function of the cochlear structure. It seems inappropriate, therefore, to continue the use of the word acoustic in the terminology of the maculae and the crista ampullaris. The walls of the utricle and saccule and of the membranous semicircular canals are formed of two very thin layers of cells, an outer connective tissue layer and an inner layer of exceedingly thin, flat epithelium. Only in the region of the maculae and the cristae do these two layers take on a marked thickening with the development of a strong basement membrane separating the two.

The *macula* of the *utricle* and *saccule* presents a structure that is fundamentally quite similar to the organ of Corti in the cochlea and the crista ampullaris of the semicircular canals in that it has as its important physiological unit a peculiar hair-bearing cell and is superimposed by a structure of epithelial origin, the otolith membrane, to which the hairs are attached. The macula consists of two layers of cells, one of neuroepithelium, the other of connective tissue cells. The latter form a thick cushion attached to the endosteum of the vestibule and made up of loosely arranged cells through which pass filaments of the vestibular nerve. It has a rich vascular network of capillaries. The *neuroepithelium* of the macula rests on a thin basement membrane by which it is sharply separated from the underlying layer of connective tissue cells. Along the margin the thick neuroepithelium passes with but a very small transition area over into the undifferentiated flat cells

FIG. 471.—Crista ampullaris, horizontal canal showing the collar or ridge below the area occupied by neuro-epithelium and the more extensive area of hair cells on one side of crista.

of the adjacent structure. The neuroepithelium is made up of two types of cells, *supporting cells* and *hair cells* or special sense cells. The supporting cells with large, oval, rather faintly staining granular nuclei lie scattered through the entire thickness of the epithelium. Most of the nuclei are

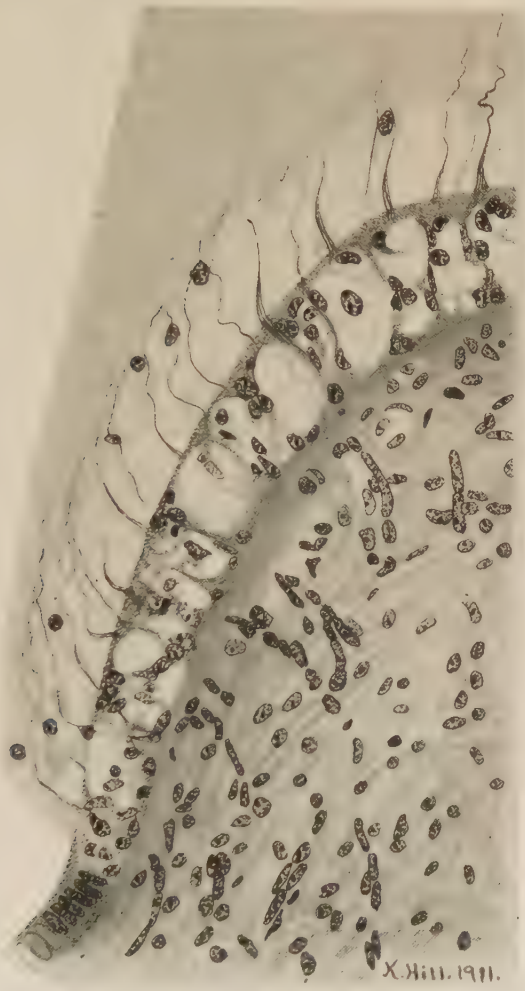


FIG. 472.—Details of crista of ampullaris showing hairs of hair cells penetrating cupola; human ear.

found in the lower half next to the basement membrane and only a few are scattered through the upper half. By some, these supporting cells have been described as having intercellular supporting fibers similar to those found in the Deiters cells of Corti's organ with processes which pass up to connect with the perforated lamina reticularis.

Fig. 473.—Preparation showing one half of crista. Note the long hairs passing from the reticular membrane cross the open space to the under surface of the cupola.

The hair cells have a more or less cylindrical shape with large oval, deeply staining nuclei which lie for the most part about the middle of the epithelial layer. Some rest on the basement membrane but a few lie here and there close under the surface plate which forms a true *reticular membrane* (Fig. 4–4). A single tapering hair proceeds from each cell. These pass out through openings in the lamina reticularis across a narrow space and become attached to the under-surface of the otolith membrane. These tapering processes are made up of a bundle of fine hairs. By some, the hairs while crossing the open space under the otolith membrane have been described as giving off thread-like processes which form a loose network of fibers that run parallel with the under-surface of the epithelium. Terminal nerve fibers from the vestibular nerve have been traced to the hair cells where they have been described as forming a fine network about the body of each cell.

The *membranous semicircular canals* consist of a narrow tube anchored along the outer or convex border of the osseous canal (Fig. 469). In the ampulla this membranous structure expands until it all but fills the osseous space. Arising from the floor of the osseous ampulla is a ridge, the *crista ampullaris*, placed transversely to the lumen so as to receive most effectively the full impact of the endolymph movements from the canal towards the utricle or in the opposite direction (Fig. 4–0). This ridge-like elevation is made up of connective tissue cells and nerve fibers and has a rich supply of capillary vessels. On the summit of the crista and extending well down on either side is a highly specialized layer of neuroepithelium over which is placed the cupola, a structure analogous to the tectorial membrane. It projects out into the endolymph of the ampulla to receive the impact of endolymph currents.

In the horizontal canal of the pig I found this layer of neuroepithelium extending further down along the canal side of the crista than on the utricular side (Figs. 4–0 and 4–1). Below the area occupied by the neuroepithelium the crista is marked by a more or less conspicuous ridge, the epithelium covering of which is made up of a single layer of tall, cylindrical cells.

The layer of neuroepithelium is separated from the underlying connective tissue cells of the crista by a well formed basement membrane perforated only by the terminal filaments of the ramus ampullaris of the vestibular nerve on their way to the hair cells. The free surface of the neuroepithelium is occupied by a *lamina reticularis* through which the hairs of the hair cells protrude. Two types of cells enter into the formation of the neuroepithelium: undifferentiated *supporting cells* packed more or less closely in the deeper layer next to the basement membrane with a few scattered sparsely through the rest of the membrane; and a peculiar hair-bearing cell lying only in the middle and outer layers, sometimes directly

under the lamina reticularis (Fig. 4–3). In structure, the *hair cells* of the crista resemble closely those found in the macula having large, oval, deeply staining nuclei. The hairs from each cell proceed as a long, single, tapering clump made up of a number of filaments quite like the hairs of the macula, pass through the openings in the lamina reticularis and crossing a narrow open space become attached to the cupola.

The *cupola* which caps the crista resembles closely the membrana tectoria and has the same function, that of receiving impulses from the endolymph and transmitting these to the hair cells where they are transformed into nerve impulses. In longitudinal sections the cupola presents a fluted appearance, the whole staining but feebly by most reagents. It was formerly not recognized as a structure different from the hairs but was supposed to

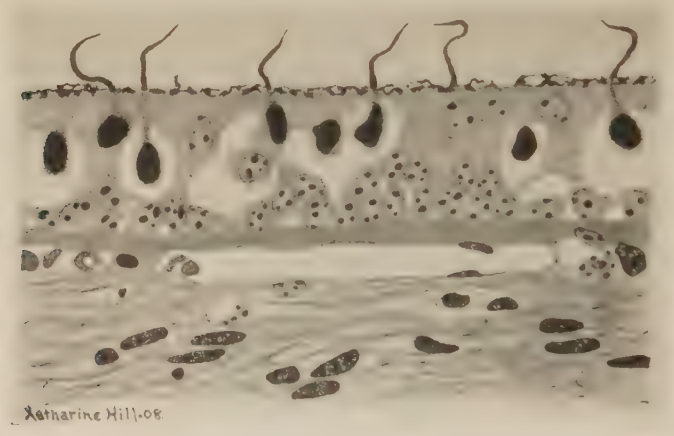


Fig. 474.—Detail of macula. Note the deeply staining nuclei of the hair cells, the hairs protruding from the reticular membrane.

be made up of the matting together of the elongated hairs acted upon by fixing agents. With the Mallory stain the cupola takes a bright blue color which readily differentiates it from the hairs which stain bright red. By using this stain I was able to demonstrate that the hairs actually penetrate the minute canals in the cupola. This relation could be shown both in sections cut longitudinally to the axis of the cupola (Fig. 4–2) and in cross sections of this structure.

The cupola is separated from the free surface of the epithelium by a narrow space which like the similar space under the otolith membrane appears to be occupied by a fluid differing somewhat from the endolymph, for, whereas the latter always remains quite clear, the fluid in this space is seen to be occupied by faintly staining globules with a finely granular substance. My preparations have usually shown, in addition to this globular

mass, small granular bodies scattered sparsely in this space, which take a deeper stain resembling cell nuclei.

The cupola, like the membrana tectoria, has a very delicate structure but in the process of fixation does not show distortion so readily from shrinkage, for the reason that it has no fixed anchorage except where it rests on the crista. It is quite probable, however, that what we see in our sections represents a structure that has undergone considerable shrinkage which has drawn the membrane up along the sides of the crista. Along its lower margin the cupola is much attenuated but in my preparations there has always been preserved a contact with the hairs which are found bent over as though dragged on by the shrunken cupola.

IV. BIBLIOGRAPHY

Alagna, L. 1909. Beitrag zur normalen und pathologischen Histologie der Ganglien des Acusticus. *Ztsch. f. Obrenheilk. u. f. Krankh. d. Luftwege*, **59**, 347.

—— 1910. Beitrag zur histologischen Technik des menschlichen Labyrinthes. *Ibid.*, **61**, 37.

Alexander, J. 1897. Zur Anatomie und Entwicklungsgesechichte der Pars inferior des Säugethierlabyrinthes. *Monatschr. f. Obrenhk.*, **31**, 276.

——— Beiträge zu Morphologie des Ohrlabyrinths. *Zentralbl. f. Physiol.*, **14**, 604.

—— 1900. Über Entwicklung und Bau der Pars inferior labyrinthi der höheren Säugetiere. *Denkschr. f. kgl. Akad. d. Wissensch.*, **70**.

—— 1901. Das Labyrinthpigment des Menschen und der höheren Säugetiere. *Arch. f. mikr. Anat.*, **58**, 134.

——— 1903*a*. Zur Entwicklungsgeschichte und Anatomie des inneren Gehörorgans der Monotremen. *Zentralbl. f. Physiol.*, **17**.

1903*b*. Entwicklung und Bau des inneren Gehörorgans von Echidna aculeata. *75 Vers. deutsch. Naturforscher. u. Arzte.*

——— 1922*a*. Zur Kenntnis der vergl. Anatomie, der Varietäten und Klinischen Bedeutung des perilymphatischen Gewebes. *Ztsch. f. Hals-, Nasen- u. Ohrenheilk.*, **3**, 167.

1922*b*. Über die Endigung des Cortischen Organs im Vorhofblindsack nebst Bemerkungen über Bau und Funktion des perilymphatischen Gewebes. *Monatschr. f. Ohren.*, **56**, 667.

Amerlinck, A. 1923. Contribution à l'étude de la membrane de Reissner et de l'épithelium de revêtement du canal cochléaire des oiseaux. *Arch. de biol.*, **33**, 301.

Beck. 1915. Die Technik der intravitalen Durchspulung zur Fixlerung des Labyrinthes fur histologische Zwecke. *Ztsch. f. Obrenheilk. u. f. Krankh. d. Luftwege*, **72**, 113.

Benjamins. 1913. Beitrag zur Kenntnis des häutigen Labyrinthes. Über eine vierte Crista acustica. *Ztschr. f. Obrenheilk. u. f. Krankh. d. Luftwege*, **68**, 101; **69**, 272.

Bielschowsky. 1907. Über die nervosen Endigungen im häutigen Labyrinth and der Säugetiere. *Arch. f. mikr. Anat.*, **71**, 22.

Chatellier, H., and Chatellier, H. P. 1925. Évolution embryologique du débouche endolymphatique chez l'homme. *Ann. d. mal. de l'oreille, du larynx*, etc., **44**, 773.

Giesswein, M. 1925*a*. Die statische Struktur des Cortischen Organes. *Ztschr. f. Hals-, Nasen- u. Ohrenhk.*, **12**, 424.

Giesswein, M. 1925*b*. Die mechanischen Verhältnisse der Basilarmembran, im besonderen der Basalwindung, und ihre Beziehungen zu akustischen Schädigungen. *Beit. z. Anat., Physiol., Path. u. Therap. d. Ohres.*, **22**, 287.

Hardesty, I. 1908. On the nature of the tectorial membrane and its probable role in the anatomy of hearing. *Am. J. Anat.*, **7**, 109.

Held. 1902–1909. *Untersuchungen über den feineren Bau des Ohrlabyrinthes der Wiebeltiere.* 2 vols. Leipz.: Tuebner.

Iwassa, M. 1925. Zytologische Studien am Gewebe des membranösen Labyrinthes des Meerschweinchens. *Folia Anat. Japan.*, **3**, 313.

Joseph. 1900. Zur Kenntnis vom feineren Bau der Gehorschnecke. *Anat. Heft.* **14**, 447.

——— 1902. Zur Histogenese des Ductus cochlearis. *Verhandl. d. österr. otol. Ges. Monatsschr. f. Ohrenheilk. u. Laryngo-Rhinol.*, **36**, 325.

Kishi. 1922. Über den Verlauf und die periphere Endigung des N. cochlearis. *Arch. f. mikr. Anat.*, **59**, 144.

Kolmer, W. Über das Verhalten der Neurofibrillen an der Peripherie. *Anat. Anz.*, **25**.

——— 1908. Über das häutige Labyrinth des Delphins. *Ibid.*, **32**, 295.

——— 1910. Über Strukturen im Epithel der Sinnesorgane. *Ibid.*, **36**, 281.

——— 1904. Über die Endigungsweise des N. octavus. *Zentralbl. f. Physiol.*, **20**, 169–171.

——— 1905. Verhalten der Neurofibrillen im Gehororgan. *Vers. deutsch. Naturforscher u. Ärzte*, **77**, 309.

——— 1907. Beiträge zur Kenntnis des feineren Baues des Gehororgans mit Berücksichtigung der Haussäugetiere. *Arch. f. mikr. Anat.*, **70**, 635.

——— 1909. Histologische Studien am Labyrinth mit besonderer Berücksichtigung des Menschen, der Affen und Halbaffen. *Ibid.*, **74**, 259.

——— 1924. Zur Kritik der Untersuchungsmethode des inneren Ohres. *Monatsschr. f. Ohrenheilk. u. Laryngo-Rhinol.*, **58**, 523.

Kompagnéetz, S. 1926. Contribution à l'étude de l'importance acoustique du pavillon de l'oreille chez l'homme. *Acta oto-laryngol.*, **9**, 110.

Kraut. 1910. Der Ductus sacculo-cochlearis (sive reuniens Henseni) bei den höheren Saugetieren und dem Menschen. *Ztsch. f. Ohrenheilk. u. f. Krankh. d. Luftwege*, **60**, 61.

Kubo, M. 1921. Experimentelle Studien über die vitale Fixation des and Gehororgans. *Organ der Japan, Oto-Rhin.-Lar. Ges.* **27**.

Kubo. 1922. Experimentelle Untersuchungen über die supravitalen und postmortalen Veranderungen der einzelnen Gewelselemente des Orhlabyrinthes. Anhang: Über die Darstellungsmethode der Cupula terminalis und über deren Hauptbestandteile. *Fubuoka ida daigaku Zassbi*, **15**.

Lagally. 1912. Beigrage zur normalen und pathologischen Histologie des Labyrhnthes. *Passows Beiträge*, **5**.

Leimgruber. 1902. Embryologisch-anatomische Studien uber die Stria vascularis. *Ztsch. f. Ohrenheilk. u. f. Krankh. d. Luftwege*, **42**, 32.

Mayschew, B. 1926. Einige histologische Beobachtungen am isolierten Kaninchenohre. *Virchow's Arch.*, **259**, 379.

Prenant, A. 1892. Récherches sur la paroi externe du limaçon des mammifères et specialement sur la strie vasculaire. *Internat. Monatschr. f. Anat. u. Physiol.*, **9**, 6.

Shambaugh, G. E. 1907. Über die Herkunft der in der tieferen Schicht der Stria Vascularis sich finden den Zellen. *Ztsch. f. Ohrenheilk. u. f. Krankh. d. Luftwege*, **53**, 301.

——— 1909. Über Bau und Funktion des Epithels am Sulcus spiralis ext. *Ztschr. f. Ohrenheilk. u. f. Krankh.*, **58**, 250.

Shambaugh, G. E. 1911. Das Verhältnis *zwischen der* Membrana tectoria und dem Cortischen Organ. *Ibid.*, **62**, 235.

——— 1912. Über den Bau und die Funktion der Crista ampullaris. *Ibid.*, **65**, 23.

——— 1923. Blood stream in the labyrinth of the ear of dog and of man. *Am. J. Anat.*, **32**.

——— 1926. The development of the membranous labyrinth. *Arch. Otolaryngol.*, **3**, 233.

Siebenmann. 1900. Neuere Untersuchungen über die Entstehung der Membrana tectoria. *Verhandl. d. deutsch. otol. Ges.*, **31**.

——— 1919. Anatomische Untersuchungen über den Saccus und Ductus endolymphaticus beim Menschen. *Beitr. z. Anat., Physiol., Pathol. u. Therap. d. Ohres, d. Nase u. d. Halses.*, **13**, 59.

Spitzer, A. 1925. Ueber die Funktion der Bogengänge des Ohrlabyrinthes. *Monatssch. f. Ohrenhk.*, **59**, 1131.

Stricht, O. 1918. The genesis and structure of the membrana tectoria and the crista spiralis of the cochlea. *Contributions to Embryology, Carnegie Inst. of Washington*, No. 227.

——— 1919. The arrangement and structure of sustentacular cells and hair cells in the developing organ of Corti. *Contributions to Embryology, Carnegie Inst. of Washington*, No. 272.

——— 1921. Origine et structure de la cupule et de la membrane otolithique. *Comptes rendus de l'association des Anatomistes.*

Wada, T. 1914. Über den histologische Bau der Stria vascularis der Schnecke. *Organ der Japan. Oto-Rhin-Lar. Ges.*, **20**, 2.

——— 1914. Über des Epithel des Sulcus spiralis externis. *Mitteilungen a. d. mediz. Fak. d. kaiserl. Univ. Tokyo*, **8**, 2.

Wittmaack. 1922. Funktionelle Bedeutung des Neuroepithels im Labyrinth. *Klin. Wchenschr.*, 2220.

——— 1924. Über die Beziehungen der Cortischen Membran zum Cortischen Organ. *Ztschr. f. Hals-, Nasen- u. Ohrenh.*, **7**, 424.

Section XXVII

THE INTERNAL ARCHITECTURE OF NERVE CELLS

CONTENTS

Section XXVII

	PAGE
I. Cell Membrane	966
II. Structure of Protoplasm in Living Nerve Cells	967
III. Chromidial Substance	968
IV. Neurofibrils	971
V. Golgi Apparatus	974
VI. Mitochondria	975
VII. Other Normal Components	976
VIII. Reaction Products	977
IX. Dynamic Polarization	978
X. Distinctive Functional Activity	981
XI. Bibliography	984

Section XXVII

THE INTERNAL ARCHITECTURE OF NERVE CELLS

E. V. COWDRY

THE all-important adjustment between an individual and his environment is determined largely by the nervous system; but a few moments' reflection will show that integration within the body is in many ways more effectively, though less rapidly, accomplished by the blood stream. If the central nervous system is mechanically destroyed, or is partly and temporarily inhibited in its action by a general anesthetic, an animal will continue to live for hours, but if his circulation is interrupted he will die almost immediately. Similarly, a completely paralyzed arm or a leg does not necessarily die but may live for years. It is the blood stream which enables the body to act as a unit in respect to respiration, assimilation, excretion and in other ways. We have seen that his tissues are protected from this environment by a coating of dead material (p. 13) and that the respiratory and digestive tracts, which communicate with the exterior, possess complete epithelial investments which serve as barriers against invading microorganisms, mechanical injury, etc. (p. 71; p. 171).

It is through localized modifications in this covering that the living cells beneath are able to receive impressions from without. The receiving cells, or "receptors," are so constructed that they respond to a wide range of stimuli; in other words, the irritability, which all living protoplasm possesses, is developed in them to a very unusual degree and in very specific ways. Yet, remarkably effective though our nervous system is, it does not, as a matter of fact, place us completely *en rapport* with our environment. Sounds occur, which we do not hear, and light waves (such as the ultraviolet) strike us, which are not perceived but which nevertheless have a profound effect upon our well-being. Indeed, the list of natural phenomena, which we would regard as non-existent if we relied only upon direct impressions received by our sense organs, is almost without end.

While the structural unit in the nervous system is thus a single cell, there is some reason to believe that the physiological unit mechanism is a reflex arc or group of cells which permits an adaptive response by other tissues of the body, that is, by the effectors, which are muscular and glandular in nature. The impulse generated at the receptor is thus transmitted elsewhere, and this power of conduction is, like irritability, a greatly enhanced property resident to a lesser degree in all living substance. To bring about this correlation the nerve cells are stretched out to a greater length than any other cells of the body. A single motor cell, for instance, may have a process several feet in length, although its greatest diameter is

less than one one-thousandth of an inch. (Volumetrically egg cells are the largest units to be found in the body—p. 1111.)

Rapid action at a distance from the exciting stimulus is characteristic of neural integration. It has come about that the chief receptors are definitely placed or anchored at the head end (cephalization) and that through millions of years the excitations have passed along fixed pathways, for otherwise they would have been futile. One could not imagine any purpose served by conduction in the opposite direction, i.e., from an effector to a receptor. In other words, there has developed a peculiar irreversibility and the individual nerve cells have become polarized (p. 0–8). In the higher animals the impulses are generally received by processes called "dendrites." They then pass to an enlargement of the cell body containing the nucleus, and thence by a single process, termed an "axon," to the cell next in series. The point of physiological contact between two nerve cells in an arc is known as the "synapse." This junction is of great functional importance and will be discussed later (p. 978).

Our actual knowledge of the peculiarly irritable and conductile protoplasm of nerve cells is very scanty. They are unique as the only elements of the adult body which it is impossible to observe more or less satisfactorily for serveral hours in the living state, because they cannot be removed without cutting their processes. Although some advances have been made (Minea, 1924), no technique has been discovered which will permit their continuous observation in tissue cultures. The neurologist can only interpret to the best of his ability the appearances seen in fixed and stained preparations on the basis of incomplete data concerning the living condition. With this great handicap it is not surprising that cytologists are concentrating their attention upon other kinds of cells which lend themselves to accurate study under controlled conditions.*

I. THE CELL MEMBRANE

The surface film of the cell is of prime importance. It may consist essentially of a monomolecular layer of protein or of lipoid; most probably of the latter.† Whether the layer is equipotential in a single cell throughout its extent we do not know (p. 0–8). The limiting film of a nerve cell certainly possesses an appreciable thickness as well as a distinct tensile strength. It is conceivable that its behavior may be modified by the localized surface concentration of material such as myelin. Indeed, it is possible to distinguish certain functional types of nerve fibers simply on the basis of the character

* The neuron theory (see Herrick, 1922) is, like the cell theory, a generalization so well founded that it is unprofitable here to discuss it.

† A fascinating description of the rôle of monomolecular layers in physical processes is given by du Noüy (1926—Monograph, Chemical Catalog Co., New York).

of their myelin sheaths, for it is well known that medullated fibers are physiologically very different from non-medullated ones. Moreover, the bodies of some cells of the spinal ganglia are protected not only by their limiting membranes, but by a continuous layer of capsule cells in addition.

II. STRUCTURE OF THE PROTOPLASM IN LIVING NERVE CELLS*

Almost all our data relate to conditions in the cell body and are in some measure to be discounted, owing to unavoidable injury to the cell processes when the tissue is excised. Supravital staining with Janus green B (Cowdry, 1912) reveals the presence of numerous mitochondria, usually granular, rod-like or filamentous in shape. Granules taking neutral red, brilliant cresyl blue, and other dyes may likewise be found. Occasional droplets of neutral fat, lipoid, glycogen (Casamajor, 1909) and of pigment are easily identified; but no definite traces of the chromidial substance, Golgi apparatus or neurofibrils can be distinguished.

The ultramicroscope, first used for nerve cells by Marinesco (1911) and later by Mott (1912), reveals myriads of brilliantly illuminated bodies which are difficult or impossible to resolve into different categories. Neither has ultraviolet photomicrography thus far proved very helpful (Stöhr, 1923). Chambers agrees with Marinesco that Brownian movement is absent ("General Cytology," p. 247). He believes, however, that cytoplasmic movements occur, although the viscosity of the cytoplasm is high, at least as compared with that of marine eggs. This is shown by the fact that it is difficult to alter the position of the mitochondria by centrifuging (Key, see Cowdry, 1918), and by Ingvar's (1923) observation that only after running the centrifuge for more than an hour is the chromidial substance gradually displaced.

The nucleus of nerve cells as seen in the living state does not differ materially from that of other cells, except that the nucleoli are unusually prominent. Their position may be easily changed by centrifuging (Ingvar). They are even influenced by gravity and in some cells naturally settle into the most dependent parts of the nuclei (Dahlgren, 1915). It is possible that the slight specific differences in the nuclei of nerve cells, as contrasted with the more pronounced variations found in other cells, may be correlated with their inability to multiply. One would not expect large differences because all somatic cells of the body are endowed with the same chromosome complex. Their specializations are initiated by environmental forces and are chiefly evident in the cytoplasm. A discussion of the nucleus-cytoplasmic ratio of nerve cells is given on page 1015.

* Useful references to neurological technique will be found in the latest edition of Lee's "Vademecum," edited by Gatenby (1928).

III. THE CHROMIDIAL SUBSTANCE (NISSL BODIES, CHROMOPHILE SUBSTANCE, TIGROID BODIES, ETC.)

Of all the elements in the nerve cell the chromidial substance has received chief attention. The technique for its demonstration in fixed tissues is simple and yields preparations which are very clear and beautiful. After preservation in alcohol, formalin, Zenker's fluid and many other fixatives,

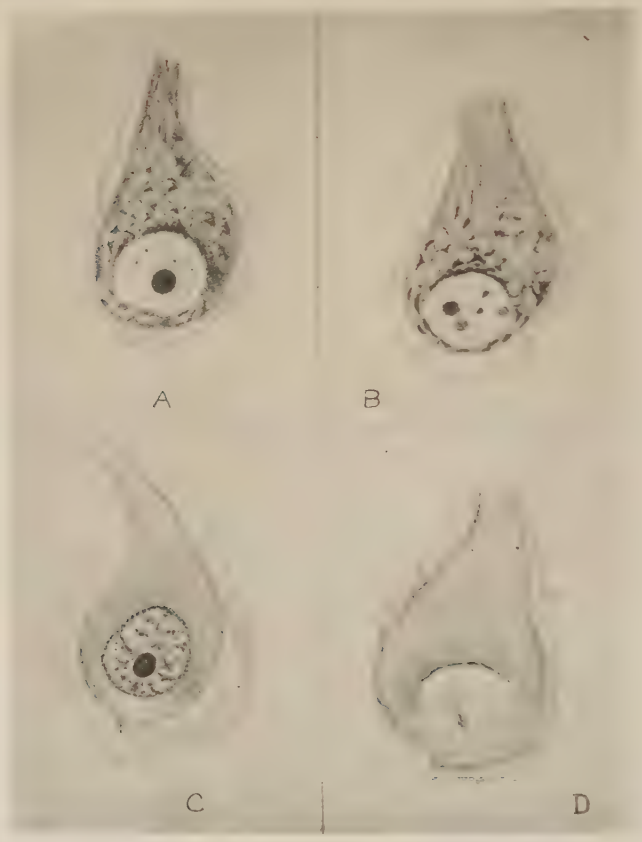


FIG. 475.—Purkinje cells from the cerebellum illustrating alterations in the nuclei and chromidial substance in fatigue: A, normal condition; B, moderate depression; C, marked depression, and D, beginning disintegration. (After Dolley, 1913.)

it occurs in the form of irregular masses of material, often of considerable size, which are colored intensely by "basic" dyes, such as methylene blue and thionin. This property has given rise to the belief that the substance is of acid nature and related to nuclear chromatin. That it is rich in iron has been shown by Mackenzie (1897) and Macallum, whose researches have

recently been confirmed by Nicholson (1923), using Macallum's hematoxylin test. The problem has been attacked from a different angle by Levene (1903), who found that the nucleic acid of the brain resembles that of other animal tissues in the character of its pyrimidine bases. In view of the practical difficulties attending the microchemical detection of phosphorus (Policard and Leulier, 1925), Scott's conclusion (1899) that this element is present in the Nissl bodies needs substantiation. A relationship between the chromidial substance and oxydases is suggested by Marinesco (1919). Unna and Gans (1914) believe it to be an albumose. It is a striking commentary upon the insufficiency of qualitative methods of microchemistry that so many years after its discovery the constitution of this material should remain practically unknown.

Reference has been made to the fact, now generally accepted, that the chromidial substance does not actually occur in such large and discrete masses in living nerve cells. In what form, then, is it found—as many minute particles visible by the ultramicroscope (Mott, 1912), or as a diffuse, optically homogeneous substratum pervading the cell body (with the exception of the axon hillock) and the dendrites (Cowdry, 1914)? The second view is in accord with van Biervliet's (1900) observation that, on its first appearance in developing nerve cells, the material is diffuse, not aggregated in masses.

Although granules of many kinds are visible with the ultramicroscope, their identification with bodies observed in similar cells when fixed and stained is extremely difficult. It is highly probable that Mott was dealing with a complex of granules including the mitochondria which we know are readily visible by the method he used. It would be difficult to find cells less favorable for ultramicroscopic examination because their large size and thickness lead to a confusing superposition of images. Perhaps the improvements in the ultramicroscope introduced lately by Barnard may facilitate resolution. Wiemann (1925) claims to have been able to secure ultraviolet light photomicrographs of the Nissl bodies (chromidial substance) in human anterior horn cells teased out in isotonic salt solution. One of the photomicrographs is reproduced in Figure 4-6. Work along this line should by all means be extended. It is possible, of course, that the masses photographed in this way do not faithfully portray conditions in the normal living cell, but point rather to a coagulation of our hypothetical, optically homogeneous deposit resulting from injury during excision of the cells or from the direct effect of the ultraviolet light, or both.

However this may be, it seems clear that the morphology of the substance may, within limits, be modified at will by the selection of fixatives. Moreover, mixtures of osmic acid and potassium bichromate do not give such sharp and clear outlines as may be obtained by using alcohol, mercuric chloride, acetic acid and other chemicals as ingredients. Nevertheless, when

standard methods are uniformly employed, with similar cells, the results are constant and entirely reliable.

The properties of the material are intimately concerned with the specific functional activity of the nerve cells containing it. To explain the significant differences in the chromidial substance, when viewed in permanent preparations of different categories of cells (pp. 999 and 1011), as well as under different functional and pathological conditions, we may fall back upon the hypothesis that the supposedly diffuse fluid material present in living cells is subject to quantitative and qualitative variations. When relatively larger

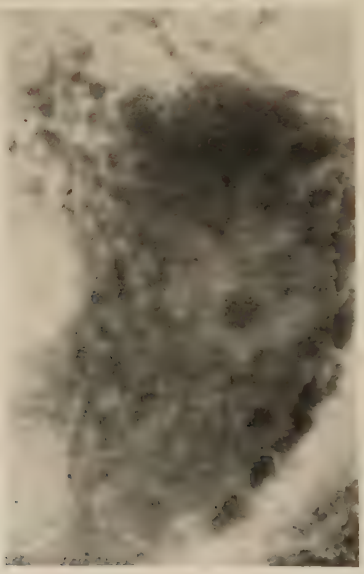


FIG. 476.—An ultraviolet photomicrograph of a still living human anterior horn cell in physiological salt solution, from a case of juvenile paralysis. The chromidial substance is seen in the form of rather large, irregular masses. Multiplication, 700 diameters. (After Wiemann, 1926.)

amounts are present, it is conceivable that the coagula resulting from fixation will be more bulky, perhaps also of greater density, and different in shape. When the amount is reduced through fatigue or injury (see p. 971), the coagula certainly become altered and decrease in size until they finally disappear. In view of the fact that the mitochondria and some other cellular components differ qualitatively in cells of different types, it is logical to assume that the chromidial substance may likewise differ qualitatively, although there is little direct evidence. Such hypothetical differences in composition might also lead to appreciable differences in the coagula.

The use of the term "chromidial substance" implies the possibility of the existence of some relationship between this material in nerve cells and

the "chromidia" described first by Hertwig in protozoa as well as several basophilic substances in a variety of tissues, but particularly in gland cells ("General Cytology," pp. 350 to 355). This is in accord with the conception that as far as the crude methods of cytology reveal them to us there are few, if any, constituents of the nerve cell distinctive and peculiar to it and without counterpart in other cells of the body. It is interesting to note that the chromidial substance is present in the proximal zone of acinus cells of the pancreas in the form of a diffuse deposit (Bensley). In this location it is not visible by dark field illumination, but its amorphous character may readily be preserved by the use of suitable fixatives, and distinct basophilic coagula may be produced at will. To the best of our knowledge, all cytoplasmic materials grouped under this general heading are rich in iron and are deposited through the interaction of the nucleus and the cytoplasm—the latter characterization is, admittedly, a vague one because it is difficult to set a limit to this interaction.

As a result of fatigue and injury the chromidial substance undergoes very characteristic changes, generally called chromatolytic, because the substance is chromatin-like and appears to dissolve (Fig. 476). Changes in sympathetic nerve cells are discussed on page 1024. Related alterations take place in the nucleus (see particularly Dolley, 1917). Stages in the disappearance of iron-containing protein following axon injury are illustrated in Figure 37 of "General Cytology." The earlier literature on experimental modifications in the chromidial substance is summarized by Barker (1901). Several later references may be added:

Centrifugation (Ingvar, 1923), fatigue and exhaustion (Dolley, 1917, and many earlier papers; Collier, 1920; Saito, 1925), gamma rays of radium (Morowoka and Mott, 1921), hypothyroidism (Mott, 1915), myxedema (Marinesco, 1924), rabies (Goodpasture, 1925), shock (Crile and Lower, 1920, Mott, 1921), thyroparathyroidectomy (Edmunds, 1912), ultraviolet light (Tello, 1911), vitamine deficiency (Kingery, 1925).

IV. THE NEUROFIBRILS

These fine, thread-like structures, as seen in fixed preparations, are illustrated in Figure 477A. Since it is very difficult to stain them satisfactorily, almost complete reliance is placed in methods of silver impregnation. It has been claimed, on very insufficient evidence, that they constitute the medium of conduction of the nervous impulse. According to Cajal, their arrangement varies in different types of nerve cells, which, if true, will constitute another qualitative difference which may be reflected physiologically (see also p. 983).

How are these appearances to be interpreted? We would naturally look in living cells for fibrils of much smaller dimensions, as the silver deposited on the surface of the supposed fibrils would tend to increase their size, or

within their interior would exaggerate them optically. But, as already stated, no fibrils, even of the most delicate type, have been seen in living nerve cells with a sufficient degree of clearness to warrant important deductions, although it is true that individual investigators have from time to time reported the existence of more or less indistinct fibrillar formations. Some of these doubtful structures may have resulted from unavoidable mechanical injury to the cells. Another possibility is that they are not in fact neurofibrils but rather mitochondria which are often quite filamentous and might easily be mistaken for them unless viewed under favorable conditions. The methylene blue and osmic acid methods, which it is claimed are helpful in the case of invertebrates, are not in my experience of any real service with the nerve cells of higher forms. The most painstaking study

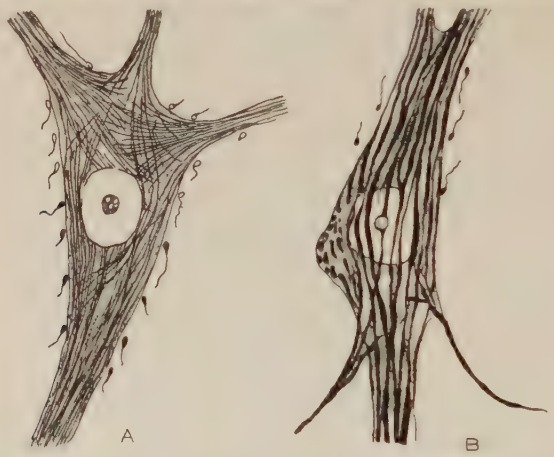


FIG. 477.—Neurofibrils during activity (A) and during hibernation (B). (After Cajal, 1906.)

with direct and oblique illumination fails to reveal the presence of any continuous surfaces of refraction, even in the parts of the cell in which the neurofibrils are most prominent in silver preparations. When the cells are crushed so that some of the cytoplasm escapes, no signs are noted of the existence of a framework or feltwork of fibrils. The granules that are microscopically visible seem to be entirely unimpeded in their movements.

But the neurofibrils must have some material basis and cannot be altogether due to technique, because silver preparations show that they appear in developing nerve cells at a definite time in relation to the assumption of functional activity and that they are composed of material radically different from the mitochondria which are the only other formed elements positively known to be invariably present in the cytoplasm (Cowdry, 1914). Once they are laid down the cells containing them are no longer capable of multiplication. This may be significant, though, like so many other phenom-

ena associated with the nervous system, it at present completely passes our understanding. When pieces of glandular tissue and of nervous tissue are placed in the same bottle and impregnated with silver in the same way, neurofibrils will come to light in the nervous tissue alone, showing that it possesses some quality not resident in gland cells. Several years ago it was found (Cowdry, 1912) that by a special technique it is possible to demonstrate at the same time in a single adult cell not only the neurofibrils and the mitochondria, but also the chromidial substance and the canalicular apparatus, which latter is in some cells identical with the Golgi apparatus (see p. 973). It is true that by this method the neurofibrils are poorly stained with fuchsin, and can be distinguished only in certain parts of the cell; but it is nevertheless clear that the peculiar material of which they are made is likewise distinct from these other two cytoplasmic components. It seems also to have a definite topographical distribution because Ingvar (1923) found that as a result of centrifuging, the whole net or feltwork of neurofibrils loosens as a unit from the cell membrane and is thus displaced.

The riddle afforded by the neurofibrils is a difficult one to solve, or even to envisage properly. We shall do well to remember that the silver methods, in which, as we have said, chief reliance is placed for their study, are of all the instruments used by the cytologist the most fickle. Yet, the neurofibrils thus brought to light have certain features difficult to explain on the basis of a mere silver deposit. Chief among these are their uniformity in diameter and smooth outlines in successful preparations.

It is not so simple to find a rough parallel for the neurofibrils in nonnervous cells as it is in the case of the chromidial substance, and, as will be seen later, of the mitochondria and of the Golgi apparatus. Intracellular fibrils are not unusual differentiations. They occur in epidermal cells and muscle cells, in connective tissue and tumor cells and in many others, but in contrast to the neurofibrils they are always readily visible in the living state, and easily stained after fixation. They usually bear a definite relation to lines of mechanical stress and strain which cannot be so clearly established in the case of neurofibrils; indeed, the nervous system, consisting of components of about the same specific gravity, surrounded by a cushion of fluid and contained within a relatively unyielding, bony cavity, should be peculiarly free from the operation of mechanical forces of this kind, after its maximum growth has been reached. Either the neurofibrils are laid down in the early stages of development, when growth is active, and retain their characteristic pattern throughout life, or else their configuration is determined by forces of some other kind, like the nervous impulse, because they are oriented parallel to its passage, the movement of ions (which may be related to it) and the transport of nutritive material. Moreover, a gradient of some kind exists in the nerve cell (p. 978) and the neurofibrils are likewise arranged parallel to it.

That the neurofibrils are in some obscure way linked up with the function of nerve cells is indicated also by the changes which they undergo in different physiological and pathological conditions. Some investigators claim that during fatigue the neurofibrils increase in number and become more slender, whereas during rest and particularly in hibernation they increase in diameter, becoming swollen (Fig. 4–8B). As a result of injury to the nerve cells containing them, they are generally supposed to fragment and disintegrate. For further details see:

A variety of conditions (Bielchowsky, 1910); centrifugation (Ingvar, 1923); hibernation (Cajal, 1906); rabies (Goodpasture, 1925); x-ray (Mondini, 1923).

V. THE GOLGI APPARATUS

The diffuse form of this "structure," or more properly speaking "material," is illustrated in Figure 4–8A. In some cases the network may be more restricted and circumnuclear in position or occupy a portion of the

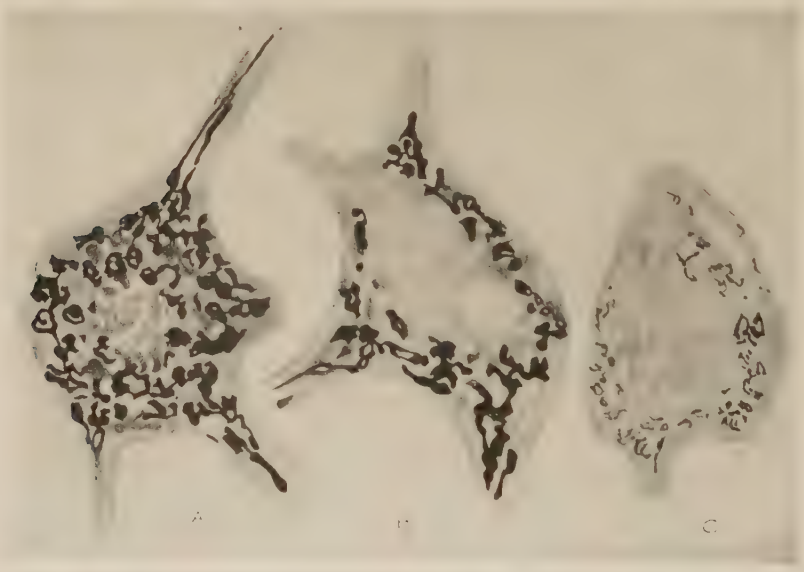


FIG. 478. Response of the Golgi apparatus to injury: (A) normal anterior horn cell; (B) retespersion in an anterior horn cell seven days after section of the sciatic nerve; (C) retespersion and retesolution in a cell of Clarke's column four days after section of the cord. (After Penfield, 1920.)

cytoplasm to one side of the nucleus. Like the chromidial substance and the neurofibrils, it has never been clearly seen in living cells. Like them also, it differs somewhat in amount and in appearance in nerve cells of different categories. It undergoes a definite evolution in form as the nerve cell

develops, and assumes distinctive functional activity. The chief methods for its investigation consist either of silver impregnation or of blackening with osmic acid, neither of which is very significant in a microchemical sense ("General Cytology," pp. 333 to 350). Some believe the material to be of lipoid nature. It is evidently of fluid consistence, because, as already stated, no resistant framework can be detected within the nerve cell and the apparatus in two cells is never of exactly the same shape, as if it varied in morphology from moment to moment.

The functional rôle of the Golgi apparatus in nerve cells, or in all others for that matter, is practically unknown. Although it remains apparently unmodified in fatigue resulting from the tetanus and strychnine convulsions, it is definitely altered by injury of the cell processes (Penfield, 1920). After section of the sciatic nerve, for instance, the network in the anterior horn cells is scattered, that is to say *retespersion* takes place (Fig. 478B). When the injury is very severe this may be followed by *retesolution*, or disintegration of the network (Fig. 478C). It is most interesting to note that, in Penfield's experiments, these alterations occurred before the chromidial substance showed any signs of change. The Golgi apparatus has been studied in a variety of physiological and pathological states:

Autolysis (Cajal, 1915); decerebration and high section of cord (Penfield, 1920); effect of cold (Da Fano, 1921*a*); electrical stimulation (Legendre, 1910); lead poisoning and senile dementia (Battistessa, 1911); nerve section (Marcora, 1910; Penfield, 1920, 1921); rabies (Cajal, 1912; Rio Hortega, 1914); section of medulla (Cajal, 1915); strychnine poisoning (Battistessa, 1911; Penfield, 1920); tetanus toxin (Penfield, 1920); transplanted ganglia (Legendre, 1910; Cajal, 1915); traumatism (Cajal, 1912, 1915); vitamine B deficiency (Da Fano, 1923*a*, *b*).

VI. THE MITOCHONDRIA

Unlike the materials already alluded to, the mitochondria unquestionably occur as such in living nerve cells, in which they may readily be demonstrated by staining with Janus green B. Their appearance is illustrated in Figures 475 and 479A. They are, as far as can be ascertained, identical with mitochondria in other cells ("General Cytology," pp. 313 to 333). In the morphological and microchemical properties of their contained mitochondria some types of nerve cells differ from others (N. C. Nicholson, 1916). When sufficient care is given to the technique, it is possible to estimate the mitochondria quantitatively with comparatively small experimental error. Working along this line, Thurlow (1917) found that the number of mitochondria per unit volume of cytoplasm is to some extent distinctive for the cells of the chief cranial nerves.

Since there is reason to believe that the mitochondria-cytoplasmic interface is of great physiological importance (Cowdry, 1926), further work

is urgently needed. A new method of measuring the extent of this interface has recently been introduced by du Noüy and Cowdry (1927). No modifications of the mitochondria in nerve cells have thus far been reported as a result of fatigue. In this respect they, in common with the Golgi apparatus, respond less readily than the chromidial substance. In experimental poliomyelitis they often retain their normal appearance, even in nerve cells in which the chromidial substance is definitely modified (McGann, 1918). Similarly, in herpetic encephalitis the mitochondria are apparently unaffected in cells which have developed characteristic nuclear inclusions

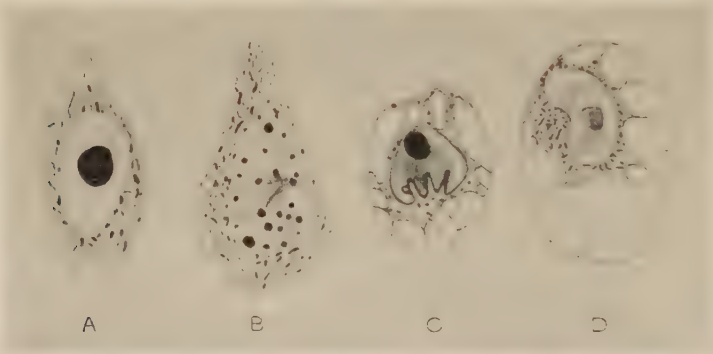


FIG. 479.—The effect of the virus of herpes on nerve cells. (A), an apparently normal cell. Note the nuclear modifications (B), and the persistence of apparently unaltered mitochondria in highly vacuolated cells (C and D). (After Cowdry and Nicholson, 1923.)

(Fig. 479B) or extensive vacuolation (Fig. 479D). But severe injury, like axon section (Luna, 1913), causes them to fragment and disintegrate. Other conditions in which they have been examined are as follows:

Beri-beri (Clark, 1914; Ma, 1925); botulism (Cowdry and Nicholson, 1924); centrifugation (Key, quoted by Cowdry, 1918); fatigue (Strongman, 1917; Saito, 1925); fatty changes (Biondi, 1915); hibernation and inanition (Rasmussen, 1919); irritation of cortex (Collen, 1914); rabies (Goodpasture, 1925); regeneration (Nageotte, 1922).

VII. OTHER NORMAL COMPONENTS

That pigments of various kinds are met with in the nervous system is indicated by such designations as *nucleus ruber* and *substantia nigra*. Neutral fat, lipoids and myelin-like droplets are of occasional occurrence in variable amounts, as are also droplets of glycogen (Casamajor, 1909); these components are usually restricted to the cell body and occur in characteristic clumps.

The fact that centrosomes are not ordinarily found in the nerve cells of adults is related to the inability of the cells to multiply, which renders

destructive lesions of the nervous system irreparable, although they may partly be cared for by the calling into action of the physiological reserve of tissue, which the nervous system possesses like the liver, kidney and other organs. With his silver methods Rio Hortega (1916) has studied the centrosomes of nerve cells extensively and has found that they undergo interesting modifications with age and in different functional states (Fig. 480).

VIII. REACTION PRODUCTS

Nerve cells, like many other cells of the body, have the faculty of producing within their interior certain materials in response to the action of specific viruses and other influences. The substances developed are highly specific and peculiar to the type of condition which calls them forth.

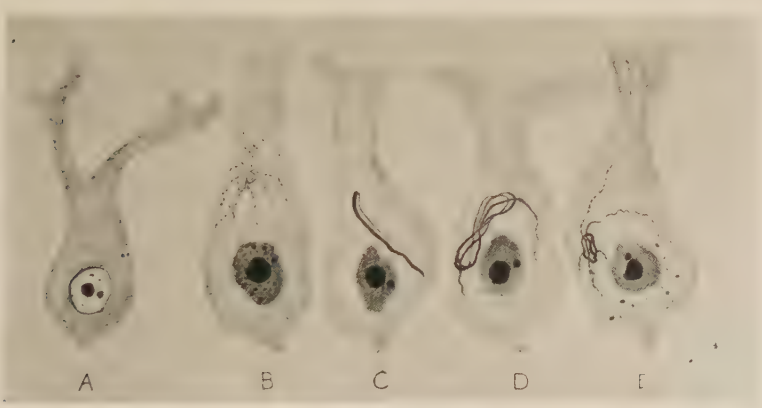


FIG. 480.—Centrosomes in Purkinje cells of the cerebellum: (A) of a boy of six years; (B) of an ox, and (C, D, E) of senile oxen. (After Rio Hortega, 1916.)

Information concerning their nature is urgently needed from the fundamental standpoint of the reaction of the cells to altered surroundings and to the penetration of unusual substances. That nerve cells are capable of producing something so specific is highly significant and if we could only understand the process of manufacture, much of the mystery of cell activity would be dissipated. The data would also serve as clues to the viruses and to the other agents involved. Among these reaction products may be cited the intranuclear herpetic inclusions (see Cowdry and Nicholson, 1923). These bodies have every appearance of being produced through the action of an enzyme upon nuclear chromatin because this material is in fact split into acid- and basic-staining fractions. The former accumulates at the center of the nucleus, while the latter moves to a position just beneath the nuclear membrane and there remains until it is completely consumed. Very important also in this category of reaction products are the Negri bodies, which

Goodpasture (1925) has recently studied by appropriate methods of technique. Many others may be mentioned, in addition, which are perhaps less specific:

Certain granules in encephalitis lethargica (Da Fano, 1921*b*) and in puerperal eclampsia (Pollak, 1906); hyaline bodies in paralysis agitans (Lafora, 1913); eosinophile granules in traumatic encephalitis (Merzbacher, 1909); "methylene blue granules" (Alzheimer, 1909); metachromatic material (Lhermitte and Radovici, 1921); nuclear inclusions in the vicinity of a glioma (Lafora, 1916); peculiar granules in "moquillo," a catarrhal infection of dogs (Rio Hortega, 1914); corpora amylacea in myotonia (Lafora, 1923), etc.

IX. DYNAMIC POLARIZATION

On the basis of the foregoing discussion we may now return to the little known mechanism which conditions the direction of passage of the nervous impulse, although this is in the first instance primarily determined by the physiological connections of the cell in question. According to the "membrane theory" (Lillie, R. S., "General Cytology," p. 224), each impulse is associated with a discharge of ions. To employ the graphic picture of Adrian (1923), " . . . the nervous impulse is a little patch of surface leakage spreading along the fibre and being sealed up again as soon as it has formed." (For a detailed discussion of nervous conduction see Davis, 1926.)

Across the synapse the conductivity is peculiar. There is a greater delay than in the cell itself and the impulse is apparently able to pass in one direction only, that is to say, it is polarized, whereas when electrically stimulated, the wave of negativity will apparently spread in both directions in the nerve cell. This irreversibility at the synapse may perhaps be in some way linked with the phenomena of "one-sided permeability" of which we should know more ("General Cytology," p. 131). The synapse differs likewise from other parts of the reflex arc in its greater susceptibility to nicotine. Its morphology lies ordinarily far beyond the limits of microscopic analysis, but the size relations are sometimes exaggerated, as with Mauthner's cell, described by Bartelmez (1920). There is in this case no evidence of direct continuity from cell to cell; on the contrary, at least three factors are involved; (1) the limiting membrane of the axon terminals of the first cell; (2) an intermediate substance; and (3) the surface of the dendrites or of the cell body of the second cell.*

Not only the synapse but also the condition of the cell membrane along which the impulse passes may be concerned. In other words, the constant passage of the impulse in one direction may of itself produce some polarization in the conducting medium or in the membrane which bounds it. Since it is logical to suppose that the properties of this membrane are in some

* Mangenot's (1926) review of synaptic connections in plants will furnish instructive collateral reading.

measure determined by the character of the cytoplasm which it limits, certain regional differentiations in the cytoplasm are of interest; but they have seldom been referred to in this relation. The contents of the dendritic termini which take up the impulse at the synapse may, it is assumed, be either optically homogeneous, like the finest of pseudopods, or contain neurofibrillar material and possibly also mitochondria, both of which apparently extend throughout the whole microscopically visible length of the cell, and are the only known morphological components so widely distributed topographically.

As one approaches the cell body, the dendrites become larger and the chromidial substance may be detected in larger amounts. When, however, the dendrites are very long, they resemble the axon in structure, inasmuch as they do not contain any detectable quantities of chromidial substance. In the cell body itself several other constituents are added, such as the nucleus, Golgi apparatus and various cytoplasmic inclusions (fat, pigment, etc.). Occasionally the Golgi apparatus is situated between the nucleus and the discharging pole, as in gland cells. According to Marinesco (1919), oxydases are present in the cytoplasm of the dendrites and cell body, and completely absent in the nuclei and in the axons; but this apparent limitation of the oxydase reaction may be simply a question of the differential penetration of the reagents. It is, however, consistent with the old concept that these are the parts of the cell chiefly concerned in assimilation, although it is difficult to conceive of a mechanism whereby the substances taken in are transported throughout the great length of the axon.*

Passing further distally, the chromidial substance is invariably lost (in amounts capable of microscopic detection) in that portion of the cell body from which the axon springs (axon hillock) and is never encountered again in the axon itself. In the axon only the neurofibrils and mitochondria may be recognized, but it has been claimed by Macdonald and also by Macallum and Menten (1905) that the axon is relatively much richer than the rest of the cell in compounds of potassium. If their test for potassium is merely a function of the penetration of the reagents, it is interesting that the said reagents should enter only, or chiefly, the axon, whereas those used in the oxydase reaction should be debarred from the axon and pass only into the dendrites and cell body, a possibility mentioned above. When the axon terminates on an effector, it is usually of appreciable size, and mitochondria as well as neurofibrils may be detected in it.

This gradient in structure seems to be related to an ill-defined gradient in metabolism, as one would expect *a priori*. The cell body with its branching dendrites offers a large surface for the absorption of nutriment, upon

* Lack of space prohibits a description of the degeneration and regeneration of nerve fibers. Useful information on this subject is given by Huber (1920) and Lewis (1923), who worked under the auspices of the War Department.

which the integrity of the axon really depends. To Tashiro (1917) we owe the positive demonstration that the resting nerve gives off CO_2 and that the amount is increased with functional activity. Herrick (1922) accepts Tashiro's experiments tending to prove "that the rate of CO_2 production is greater in that portion of a nerve fiber which lies nearer to the source of the stimulus than in a similar portion of the same nerve fiber further from the receptive end and nearer to the discharging end." He cites, in confirmation, Child's observations (1921) "that different parts of the nerve fiber show differences in susceptibility to certain poisons, corresponding to the differences in rate of oxidation of their substance." The crucial element in these estimations is their degree of accuracy from a quantitative point of view. For further observations and a critical discussion of the subject, see Parker's recent paper (1925).

In an attempt to explain the development of dynamic polarization, Kappers (1921) has built up an ingenious theory based on the action of bioelectric forces. This has been supported experimentally by Ingvar (1920)

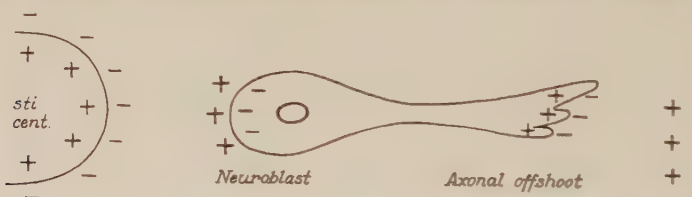


FIG. 481.—Distribution of electric potentials in a neuroblast during the outgrowth of its axon. (After Kappers, 1921.)

who applied a weak galvanic current to tissue cultures made by Harrison's method. He found that: "The galvanic current has a directing influence upon the cell and fiber outgrowth in the cultures, so that this occurs almost entirely along the lines of force in the galvanic field. Whereas in the control preparations the outgrowth occurs in all directions; cell movements under the influence of a galvanic current take place toward the anode and the cathode. The cell processes growing toward the anode show morphological differences from those growing toward the anode."

The accompanying diagram (Fig. 481) illustrates the distribution of electrical potentials. The axon process is the first to develop and grows toward the anode (positive pole). This, Kappers believes, is conditioned to some extent by its richness in positive potassium ions, accepting, as he does, the above-mentioned microchemical tests made by Macdonald and by Macallum and Menten (1905). Later the dendritic process grows out in the opposite direction toward the stimulation center (the cathode or negative pole). He emphasizes the fact that this extension occurs coincidentally with the appearance in this pole of the cell of the Nissl or chromidial substance ("a

compound of nucleic acid with iron"). This shifting of colloidal material in a galvanic current is in conformity with experimental studies on cataphoresis. The whole theory is an attractive one, capable of explaining at least tentatively many interesting points in the architecture of the nervous system, such as the spatial orientation of the cells and processes, and the frequent presence of one axon as compared with several dendrites. An interesting investigation into the morphology of anterior horn cells, as determined by forces of this kind, has recently been made by Kraus (1926).

X. DISTINCTIVE FUNCTIONAL ACTIVITY

In concluding, we may attempt to synthesize warily. It will be apparent from the foregoing discussion that the several structural components of the nerve cell have usually been considered individually and separately. As a result, there has been but little correlation, notwithstanding the fact that we all recognize that the cell is a unit in which all the vital processes are mutually dependent and take place in harmony. No element can logically be described as peculiarly living or dead, as has been emphasized by the biochemist, Hopkins. The reason for this one-sided approach is that neurology is a science pursued chiefly by specialists in which no cooperation with workers in related fields has been effected on a scale comparable to that which has proved so productive in other divisions of medical science. Coupled with this is the fact that the nerve cell is particularly difficult to study, for reasons already enumerated. No marked changes occur in its form, as in muscle cells, or large discharges of material, as in gland cells. If, for example, a section of the cerebellum be examined in a normal resting animal, the Purkinje cells will be found to present striking differences in appearance presumably indicative of differences in functional activity, so that it is difficult to establish a convenient starting point for experimentation. They often act individually, not synchronously, in groups, like gland cells or muscle cells.

Only recently has the conducting medium been shown to take up oxygen and give off carbon dioxide like other tissues, and the temperature coefficient of velocity of the impulse is still in doubt (Hill, 1921; Parker, 1925). With the onset of functional activity, there seems to be a swelling of the cell (Herrick, 1922; see, however, Kocher, 1916), followed by a shrinkage. If the activity is continued to the extent of marked fatigue, the chromidial substance is reduced in amount (Fig. 476) and it is possible that the neurofibrillar material is modified, despite our lack of certain information. As a result of severe injury these components together with the mitochondria and Golgi apparatus are all altered in the direction of loss of characteristic form and disappearance of material, but the interdependence of the changes is now known. At the same time, the permeability of the cell is modified, as

illustrated by the fact that it now takes up vital dyes. Chronic injury of low-grade intensity is sometimes manifested by the heaping up of pigment (p. 1023) and by the accumulation of other so-called "inclusions." Of the structural changes during sleep and under hypnotic influence we know almost nothing (see, however, Herrick's summary, 1922).

"The comparison of a nerve impulse to the burning of a chain of gunpowder, though not very close, is nevertheless in many ways suggestive" (Parker, 1925). To explain the relatively very small temperature coefficient Parker suggests that the nerve, like muscle, may "store carbonates from which with a minimum of heat production CO_2 may be liberated by an acid formed in the nerve." Herrick (1922) believes that the activity of the nerve cell is of the explosive type, and that this quality is largely dependent upon the presence in it of large amounts of chromidial substance ("chromophile substance") which is presumably oxidizable throughout its entire mass almost instantaneously. In other words, "the organization of the nerve cell is such that a very small stimulus may liberate a large amount of energy with explosive suddenness."

Some gland cells, which exhibit alternating periods of elaboration and discharge of secretion, are, in like manner, provided with an abundant supply of chromidial substance (p. 243), but unfortunately a detailed comparison between this material and that found in nerve cells has not been made. When, however, we attempt to extend this comparison to the striated muscle cells of vertebrates (p. 769), we find that although a condition of rest may be suddenly followed by one of intense activity, cytoplasmic material resembling the chromidial substance is conspicuous by its absence.

It is generally supposed that the impulse transmitted by different types of nerve cells is qualitatively the same, though different in the receptors and effectors involved. This may or may not be the case. Many protoplasmic activities are, like the nervous impulse, accompanied by electrical changes ("action currents") but they may be, for example, contractile or secretory in character and thus differ in kind. Nerve cells in different categories differ not only in respect to their size and the morphology of their processes, but also in their internal structure. Indeed, their differential properties are almost legion.

Attention has already been called to variations in the chromidial substance, mitochondria, Golgi apparatus, degree of myelination and other properties (p. 908). There is evidence that the arrangement of neurofibrils is in some measure indicative of different cell types (p. 1011). Some nerve cells elaborate quite specific pigments, while others remain throughout life devoid of pigment. The chrome-silver methods of impregnation discovered by Golgi are often surprisingly elective, picking out, for some unknown reason, special cells and leaving the remainder practically invisible. Certain groups of cells, or areas in the brain, differ from others in their iron

content (Gans, 1923) and in the possession of special affinities for organic and inorganic poisons. In various mental disorders special parts of the brain become affected in definite sequence for which no adequate explanation has ever been offered. The tendency of the Negri bodies to develop first in the cells of the hippocampus can only mean that these cells differ in some obscure way from the others, not readily explainable on the basis of location or of a peculiarity in blood supply. The application of indicators to the central nervous system, in accordance with methods chiefly elaborated by Rous (1925), may show that the different centers, like some other tissues of the body, are adapted to function at certain definite hydrogen-ion concentrations. It is possible that attributes such as these, real and as yet largely imaginary, are reflected physiologically. Certainly if gland cells differed so materially in structure and in response, we would not expect to find their secretion identical. It is, in fact, accepted that the rhythm of discharge and the rate of conduction differ in different types of nerve cells (Herrick, 1925).

Whether nerve cells possess other distinctive activities in addition to those of irritability and conduction we do not know. It is commonly said that all cells of the body are potentially internal-secreting, and claims have been forwarded from time to time of the discovery in nerve cells of secretory antecedents. The evidence in the case of certain lower forms (Speidel, 1922) is more convincing than it is for man and mammals. The recent observation by Minot that extracts of the liver possess a substance very active physiologically, the existence of which was previously wholly unsuspected, is a case in point and indicates that we must keep an open mind. The medulla of the suprarenal glands is of nervous origin and produces an internal secretion (p. 627) which is extremely powerful. It is possible that nerve cells which are less modified may elaborate a substance of kindred nature, as suggested by Macallum.

From another angle, also, we have indirect information, because none will deny the observation that organic disease of the nervous system exercises a profound effect upon the rest of the body, and vice versa. Whether this is a matter of perverted metabolism, lack of tone, the discharge of specific substances or of a whole interdependent chain of forces as yet undreamed of, we cannot tell. The rôle of the nervous system in the initiation of exophthalmic goiter, for example, though accepted as an etiological agent, is unknown in character. The function of the thyroid may conceivably be deranged by the receipt of unusual nervous impulses, or by the action of substances of nervous origin, arriving via the blood stream, or in still other ways. Recent investigations on the behavior of choline as a hormone (Mulinos, 1926) may be of some significance in this connection if it is found that any considerable amount of this substance is liberated by the disintegration of the phospholipins of the brain, as may very well be

the case. But if such other activities do exist, it is natural to suppose that they are overshadowed by the specific functions of the nervous elements—irritability and conduction, perhaps also memory (Mathews, 1925)—more completely than in other tissues because differentiation has here been carried to such an extreme.

XI. BIBLIOGRAPHY

Adrian, E. D. 1923. Disorders of function in the neurone. *Proc. Roy. Soc. Med.* (Sect. Neurol.), **16**, 55.

Alzheimer, A. 1909. Beiträge zur Kenntnis der pathologischen Neuroglia und ihrer Beziehungen zu den Abbauvorgängen im Nervengewebe. *Histol. u. histopath. Arb. u. d. Grossbirnrinde*, Jena, **3**, 401.

Barker, L. F. 1901. *The nervous system and its constituent neurones.* New York.

Bartelmez, G. W. 1920. The morphology of the synapse in vertebrates. *Arch. Neur. and Psychiat.*, **4**, 122.

Battistessa, P. 1911. Sulle alterazioni dell'apparato reticolare interno di Golgi nelle cellule nervose dei gangli intervertebrali nella demenza senile. *Note e rit. di psichiat.*, **4**, 384.

— 1912. Sulle alterazioni dell'apparato reticolare interno delle cellule nervose dei gangli spinali in seguito ad avvelenamento da piombo e stricnina. *Riv. ital. di neuropat.*, **4**, 345.

Bielchowsky, M. 1910. *Handbuch d. Neurologie* (Lewandowsky), **1**, 45.

van Biervliet. 1900. La substance chromophile pendant le cours de développement de la cellule nerveuse. *Le Névraxe*, **1**, 33.

Biondi, G. 1915. Ueber Fettphaneroses in der Nervenzelle. *Virchow's Archiv*, **220**, 222.

Cajal, S. R. 1906. Structure et connexions des neurones (Nobel Prize Address). *Arch. di Fisiologia*. **5**.

1912. Fórmula de fijación para la demonstración fácil del aparato de Golgi, etc. *Trab. d. lab. de invest. biol.*, Univ. de Madrid, **10**, 209.

— 1915. Alcunas variaciones fisiológicas y patológicas del aparato reticolar de Golgi. *Trab. d. lab. de invest. biol.*, Univ. de Madrid, **14**, 107.

Casamajor, L. 1909. Zur Histochemie der Ganglienzellen der menschlichen Hirnrinde. *Arb. a. d. Neurol. Inst.*, Wien, **18**, 101.

Child, C. M. 1921. *The origin and development of the nervous system from a physiological viewpoint.* Univ. of Chicago Press.

Clark, E. 1914. Regeneration of medullated nerves in the absence of embryonic nerve fibers, following experimental non-traumatic degeneration. *J. Comp. Neurol.*, **24**, 61.

Collier, W. D. 1920–21. The experimental production of functional hypertrophy in the nerve cell. *J. Med. Res.*, **42**, 439.

Collin, R. 1914. Sur les mitochondries extraneuronales dans l'écorce cérébrale irritée. *Compt. rend. Soc. de biol.*, **67**, 591.

Cowdry, E. V. 1912. The relations of mitochondria and other cytoplasmic constituents in spinal ganglion cells of the pigeon. *Intern. Monatsschr. f. Anat. u. Phys.*, **29**, 473.

1914. The comparative distribution of mitochondria in the spinal ganglion cells of vertebrates. *Am. J. Anat.*, **17**, 1.

1918. The mitochondrial constituents of protoplasm. *Contrib. Embryol.*, Carnegie Inst. Wash., **8**, 39.

——— 1926. Surface film theory of the function of mitochondria. *Am. Nat.*, **60**, 157.

Cowdry, E. V., and Nicholson, F. M. 1923. Inclusion bodies in experimental herpetic infection of rabbits. *J. Exp. Med.*, **38**, 695.

——— 1924. An histological study of the central nervous system in experimental botulinus poisoning. *Ibid.*, **39**, 827.

Crile, G. W., and Lower, W. Z. 1920. *Surgical shock.* Phila.: W. B. Saunders Co. 272 pp.

Da Fano, C. 1921*a*. Changes of Golgi's apparatus in nerve cells of the spinal cord following exposure to cold. *J. Nerv. and Ment. Dis.*, **53**, 353.

——— 1921*b*. The histo-pathology of epidemic (lethargic) encephalitis. *Brit. Med. J.*, **1**, 153.

——— 1923*a*. The canalicular apparatus within nerve cells of the spinal cord, spinal and sympathetic ganglia in vitamin B deficiency. *J. Physiol.*, **57**, liv.

——— 1923*b*. Golgi's apparatus and Nissl's substance of nerve cells of the spinal cord and ganglia in deficiency diseases. *Ibid.*, **57**, lvii.

——— 1923*c*. Herpetic meningo-encephalitis in rabbits. *J. Path. and Bact.* **26**, 85.

Gans, A. 1923. Iron in the brain. *Brain*, **46**, 128.

Goodpasture, E. W. 1925. A study of rabies, with reference to a neural transmission of the virus in rabbits, and the structure and significance of the Negri bodies. *Amer. J. Path.* **1**, 547.

Herrick, C. Judson. 1922. *Introduction to neurology.* Phila.: W. B. Saunders Co. 394 pp.

——— 1924. *Neurological foundations of animal behavior.* N. Y.: Henry Holt Co. 334 pp.

Hill, A. V. 1921. The temperature coefficient of the velocity of the nervous impulse. *J. Physiol.*, **54**, 332.

Huber, G. C. 1920. Repair of peripheral nerve injuries. *Surg., Gynec. & Obst.*, **30**, 464.

Ingvar, S. 1919–20. Reaction of cells to the galvanic current in tissue cultures. *Soc. Exp. Biol. and Med.* **17–18**, 198.

——— 1923. Centrifugation of the nervous system. *Arch. Neurol. & Psychiat.*, **10**, 267.

Kappers, C. U. Ariens. 1921. On structural laws in the nervous system: The principles of neurobiotaxis. *Brain*, **44**, 125.

Kingery, H. M., and Kingery, J. B. 1925. A study of the nervous tissues of albino rats fed on vitamine-free diet. *Anat. Rec.*, **29**, 364.

Kocher, R. A. 1916. The effect of activity on the histological structure of nerve cells. *J. Comp. Neur.*, **26**, 341.

Kraus, W. M. 1926. Human adult and embryonic anterior horn cells. *Arch. Neurol. and Psychiat.*, **15**, 686.

Lafora, G. R. 1913*a*. Nuevas investigaciones sobre los cuerpos amiláceos del interior de las células nerviosas. *Trab. d. lab. de invest. biol.*, Univ. de Madrid, **11**, 29.

——— 1913*b*. Contribución á la histopatologia de la parálisis agitante. *Ibid.*, **11**, 43.

——— 1916. Modifications des cellules névrogliques et des cellules nerveuses dans un gliome. *Ibid.*, **14**, 103.

——— 1923. Les myoclonies et les corps amylacés dans les cellules nerveuses. *Rev. neurol.*, **2**, 399.

Legendre, R. 1910. Recherches sur le réseau interne de golgi des cellules des ganglions spinaux. *Anat. Anz.*, **36**, 207.

Levene, P. A. 1903. On the chemistry of the chromatin substance of the nerve cell. *J. Med. Res.*, **10**, 204.

Lewis, D. 1923. Peripheral nerve problems. *Bost. Med. and Surg. J.*, **188**, 975.

Lhermitte, J., and Radovici, A. 1921. Étude sur la dégeneration basophile metachromatique des fibers et des cellules du cerveau et de la moëlle épinière dans l'encéphalite épidémique. *C. rend. Soc. de biol.*, **84**, 931.

Luna, E. 1913. Sulle modificazioni dei plastosomi delle cellule nervose nel trapianto ed in seguito al taglio dei nervi. *Anat. Anz.*, **44**, 413.

Ma, W. C. 1925. A study of the mitochondrial elements of spinal ganglion cells of beriberi fowls. *Am. J. Anat.*, **36**, 215.

Macallum, A. B., and Menten, M. L. 1905. Distribution of chlorides in nerve cells and fibers. *Proc. Roy. Soc.*, **76**, (B), 217.

McGann, G. F. 1918. A study of mitochondria in experimental poliomyelitis. *J. Exp. Med.*, **27**, 205.

Mackenzie, J. J. 1897. Investigation in the microchemistry of nerve cells. *Report of the Brit. Assn. for the Adv. of Science*, Toronto, August 23, 822.

Mangenot, G. 1926. Communications intercellulaires et synpases. *Bull. d'Hist. Appl.*, **3**, 142.

Marcora, F. 1910. Sulle alterazioni dell'apparato reticolare interno delle cellule nervose motorici consecutive a lesioni dei nervi. *Riv. di patol. nerv.*, **15**, 393.

Marinesco, G. 1911. Études ultramicroscopiques des cellules des ganglions spinaux. *Compt. rend. Soc. de biol.*, **70**, 1057.

——— 1919. Études histologiques sur les oxydases et les peroxydases. *Ibid.*, **82**, 258.

——— 1924. Contribution à l'étude des lésions du myxodème congénital. *L'encéphale*, **19**, (5), 265.

Mathews, A. P. 1925. *Physiological chemistry*. N. Y.: William Wood and Co. 1232 pp.

Merzbacher, L. 1909–10. (Traumatic encephalitis.) *Histol. u. histopath. Arb.*, **3**, 139.

Minea, J. 1924. Culture in vitro de cellules nerveuses isolées. *C. rend. Soc. biol.*, **90**, 1353.

Mondini, U. 1923. Richerche sul reticolo fibrillare endocellulare in seguito ad azione dei raggi x, e di raggi x combinati con il freddo. *Riv. di patol. nerv.*, **28**, 14.

Morowoka, T., and Mott, F. W. 1921. Histological examination of the brains of animals exposed to the Gamma rays of radium. *Rep. Med. Res. Coun.*, **62**, 131.

Mott, F. W. 1912. The biophysics and biochemistry of the neurone. *Brit. Med. J.*, **2**, 780.

——— 1915. Microscopic examination of the central nervous system in three cases of spontaneous hypothyroidism in relation to a type of insanity. *Proc. Roy. Soc. Med.*, **8** (Sect. of Psychiat.), 58.

——— 1921. The neurological aspects of shock. *The Lancet*, **1**, 519.

Mulinos, M. G. 1926. Studies on cholin as a motor hormone for the alimentary tract. *Am. J. Physiol.*, **77**, 158.

Nageotte, J. 1922. *L'organisation de la matière dans ses rapports avec la vie*. Paris: Librairie Felix Alcan.

Nicholson, F. M. 1923. The changes in amount and distribution of the iron-containing proteins of nerve cells following injury to their axones. *J. Comp. Neurol.*, **36**, 37.

Nicholson, N. C. 1916. Morphological and microchemical variations in the mitochondria in the cells of the central nervous system. *Am. J. Anat.*, **19**, 329.

du Noüy, P. Lecomte. 1926. Surface equilibria of organic and biological colloids. *Am. Chem. Soc. Monographs*, Chem. Catalog. Co.: New York.

——— and Cowdry, E. V. 1927. Cytological measurements to test du Nony's thermodynamic hypothesis of cell size. *Anat. Rec.*, **34**, 313.

Parker, G. H. 1919. *The elementary nervous system*. Phila. J. B. Lippincott Co.

——— 1925. The production of carbon dioxide by nerve. *J. Gen. Physiol.*, **7**, 641.

Penfield, W. G. 1920. Alterations of the Golgi apparatus in nerve cells. *Brain*, **43**, 290.

——— 1921. The Golgi apparatus and its relationship to Holmgren's Trophospongium in nerve cells. Comparison during retispersion. *Anat. Rec.*, **22**, 57.

Policard, A., and Leulier, A. 1926. Étude critique sur les méthodes de caractérisation histochimique du phosphore. *Bull. d'Hist. Appl.*, **2**.

Pollak, E. 1906. Die Befunde am Zentralnervensystem bei der puerperalen Eklampsie. *Arb. neurol. Inst. Wien. Univ.*, **13**, 1.

Rasmussen, A. T. 1919. The mitochondria in nerve cells during hibernation and inanition in the woodchuck (Marmota monax). *J. Comp. Neur.*, **31**, 37.

Rio Hortega, P. del. 1914. Alteraciones del sistema nervioso central en un caso de moquillo de forma paralitica. *Trab. d. lab. de invest. biol.*, Univ. de Madrid, **12**, 97.

——— 1916. Estudios sobre el centrosome de las cellulas nerviosas y neuroglicas de los vertebrados, en sus formas normales y abnormales. *Trab. d. lab. de invest. biol.*, Univ. de Madrid, **14**, 117.

Rous, P. 1925. The relative reaction within living mammalian tissues. *J. Exper. Med.* **41**, 739.

Saito, T. 1925. Studies on histological changes in the nerve cells resulting from overactivity. *Orienta Bol. Neurobiol.*, **1**, 1.

Scott, F. H. 1899. On the structure, micro-chemistry and development of nerve cells, with special reference to their nuclein compounds. *Trans. Canadian Inst.*, **6**, 405.

Speidel, C. C. 1922. Further comparative studies in other fishes of cells that are homologous to the large irregular glandular cells in the spinal cord of the skates. *J. Comp., Neurol*, **34**, 303.

Stöhr. 1923. Zur Architektur der Nervenzellen im ultravioletten Mikrophotogramm. *Verb. d. Anat. Ges.*, **32**, 154.

Strongman, B. T. 1917. A preliminary experimental study on the relation between mitochondria and the discharge of nervous activity. *Anat. Rec.*, **12**, 167.

Tashiro, S. 1917. *A chemical sign of life.* Univ. of Chicago Press.

Tello, F, 1911. Algunas observaciones con los rayos ultravioletas. *Trab. d. lab. de invest. biol.*, Univ. de Madrid, **9**, 111.

Thurlow, M. DeG. 1917. Quantitative studies on mitochondria in nerve cells. *Contrib. Embryol.*, Carnegie Inst. Wash., **6**, 35.

Unna, G., and Gans, O. 1914. Zur Chemie der Zelle. IV. Die Nisslkörper, *Berl. klin. Wchnschr.*, **51**, 444.

Wiemann, W. 1925. Studien am Zentralnerven system des Menschen mit Mikrophotographie im ultra violetten Licht. *Zeit. f. d. ges. Neurol. u. Psychiat.*, **98**, 347.

Section XXVIII

THE GENERAL RELATION OF HISTOLOGICAL CHARACTER TO FUNCTION IN MAMMALIAN NEURONS

CONTENTS

Section XXVIII

PAGE

I. Two Portions of Neuron, and Three Ways in which They may be Related to Course of Nervous Impulse 993

II. Relation of Cell Body and Dendrites to Phenomenon of Central Fatigue 995

III. Functional Significance of Histological Characters in Peripheral Neurons 998

IV. Relation of Afferent and Efferent Systems to Correlation and Coordination, and Relation between Histological and Functional Characters in Various Types of Correlating and Coordinating Neurons 1001

V. Conclusion 1004

VI. Bibliography 1005

Section XXVIII

THE GENERAL RELATION OF HISTOLOGICAL CHARACTER TO FUNCTION IN MAMMALIAN NEURONS

E. F. MALONE

ALTHOUGH all neurons are concerned in the same general process of excitation-conduction, they exhibit great variation in histological character, and such histological modifications are beyond doubt associated with corresponding functional differences. All neurons possess a high degree of irritability which insures not only a high metabolic activity but also a delicate adjustment of immediate or intraneuronic response to stimulus. This immediate response involves excitation-conduction, through which excitation of distant structures is effected. Thus the irritability common to all neurons involves within the limits of each neuron a local activity which is not terminal but which on the contrary serves merely to initiate activities within other structures. Moreover, the stimuli which a neuron receives depend in part upon its own previous activities. Within the central nervous system the immediate response and the stimulus are merely two aspects of the same activity, since each immediate response to a stimulus serves as a stimulus to initiate other responses in succeeding structures. Due to differences in anatomical connections different groups of neurons are acted upon by different groups of stimuli and upon this fact depends the functional and histological specialization of the various neuron types. Since the intraneuronic activity of all neurons is fundamentally similar and since every neuron directly or indirectly effects a widely distributed stimulation throughout the nervous system, one would expect different neuron types to exhibit certain functional and histological characters in common; later it will be shown that this interrelation of neuron types does exist. It is a significant fact that development continues after neurons have begun to function, and that histological and functional development proceed simultaneously (Coghill, 1924).

Leaving out of consideration such activities as are concerned merely in maintaining the specific protoplasm of the neuron, the function of a neuron has two distinct aspects. One can consider its function to consist of those local activities which under given conditions occur within the limits of the neuron itself, and it is largely this aspect of function to which corresponds the neuron's histological character. Or one can also consider the function of a neuron from the standpoint of its connections, and the significance of these connections presents in turn two different aspects. In the first place, any connection of a neuron may be considered from a purely topographical standpoint, emphasis being placed upon the location of the associated struc-

ture without reference to its function. Such purely topographical differences in connection have little relation to the neuron's histological character except in so far as they involve axon length. But every connection of a neuron involves not only the location of the associated structure but also the manner in which this structure is employed in the activities of the organism. In other words, the function of a neuron involves its individual local or intraneuronic activity and also the neuron's topographical and specific functional relations to the entire organism. While a neuron's histological character corresponds to its local internal activities and while the latter depend upon the activities of associated structures (direct stimuli from preceding and indirect stimuli from succeeding structures), it by no means follows that every neuron in the same functional mechanism will receive identical stimuli. For a neuron's functional and histological character depends not only upon the nature of the mechanism of which it is a part but also upon its manner of employment in this mechanism. Radically different mechanisms may in part be practically identical, while mechanisms closely related with reference to the organism's activities may differ greatly in details of operation. Unfortunately most of the functional characters of a neuron can be studied only through the activities of the mechanisms in which it is found, but the presence of the same histological type of neuron in widely different mechanisms permits a certain degree of insight into the general uses of such a neuron, as does the occurrence of different neuron types in mechanisms whose activities differ only to a limited extent.

It is necessary to discuss certain neurological terms. Neurons may be divided into two general groups, "somatic" and "visceral." "Somatic" neurons are employed in the adjustment of the organism to the outer world, while "visceral" neurons adjust the internal processes of the organism. The distinction between "somatic" and "visceral" neurons is valuable with respect to efferent neurons, since the "somatic" supply skeletal muscle, while the "visceral" supply smooth muscle and glands. But to apply these terms to two distinct subdivisions of the nervous system employed in external and internal activities of the organism is of doubtful value; for the internal activities of the organism and those activities which relate it to the outer world are not sharply separated, since external stimuli may affect internal (visceral) activities and the latter may affect somatic activities.

Another valuable but not altogether satisfactory distinction is implied by the terms "exteroceptive," "interoceptive" and "proprioceptive," terms applied to afferent neurons conducting impulses initiated by activities within the outer world, activities affecting the mucous membranes and activities within the mesodermal structures respectively. The distinction between "exteroceptive" and "interoceptive" senses is imperfect, for the mucous membranes are continuous with the skin and form a surface which is itself in contact with the outer world. Where this surface is intimately related to the outer world (nose, tongue) sensation is well developed, while in its more remotely related portions "interoceptive" sensation is poorly developed. In general, both "exteroceptive" and "interoceptive" senses relate the organism to the outer world and both are concerned in "visceral" as well as in "somatic" activities. "Proprioceptive" neurons are stimulated by activities within the organism itself; these activities may be either somatic

or visceral and may involve either relations within the organism or else the spatial relation of the organism to the outer world.

While the distinction between "somatic" and "visceral," between "exteroceptive," "interoceptive" and "proprioceptive" is, within limits, of practical value, any attempt at a thorough subdivision of the nervous system upon the basis of such distinctions results inevitably in failure. For the organism is a unit and the unifying action of the nervous system involves intimate relations between all types of activity, whether somatic or visceral and whatever the source of the initiating stimuli. Thus the fundamental unifying activity of the nervous system renders of necessity any subdivision into distinct functional systems impossible. But bearing in mind this limitation, such attempts at subdivision are instructive.

The terms "correlation" and "coordination" are used by Landacre to distinguish two types of nervous adjustment (Herrick, 1924). "Correlation" involves those combinations of afferent impulses within sensory centers which form the basis or plan of appropriate adaptive responses. "Coordination" is associated with the efferent system and consists of those adjustments which, employing anatomically fixed arrangements of neurons, effect the necessary steps in the execution of the plan determined by the process of "correlation." The essential difference between "correlation" and "coordination" is that correlating adjustments are easily modified by slightly changed external or internal conditions, while coordinating adjustments are more stable. In general, "correlation" involves sensory (afferent) centers and "coordination" motor (efferent) centers, but, as will appear later, this distinction does not always hold; in this section these two terms designate two types of nervous adjustment, one of which is labile and the other stable or stereotyped.

1. THE TWO PORTIONS OF THE NEURON, AND THE THREE WAYS IN WHICH THEY MAY BE RELATED TO THE COURSE OF THE NERVOUS IMPULSE

Neurons cannot be divided into sharply separated histological types, for each type presents a histological complex of which certain individual characters may appear in other neuron types (Malone, 1923*b*). Thus a large cell body may be associated with a long or short axon and with different types of dendrites and of internal structure. Every neuron consists of two portions which differ widely in histological appearance and, as will be shown, also in function. One of these two portions is the cell body, together with any structures which are merely direct continuations of essentially the same type of cytoplasm as that contained in the greater part of the cell body; these structures, when they occur, and these only should be termed dendrites and together with the cell body constitute one

portion of the neuron. The other portion is the axon and possibly the axon hillock, and for convenience the term axon will be considered to include this portion of the cell body. Those structures known as "physiological dendrites" (sensory fibers of nerve trunks) are anatomically axons and possess the same basic function as do axons, for they are highly specialized conductors obeying the all or none law. All axons can conduct efficiently in either direction, and the fact that in certain axons the normal direction of conduction and the metabolic gradient is towards the cell body is a physiological consideration by no means fundamental with reference to the axon itself. In other words, the direction of conduction in such axons is under existing conditions to be expected and the significance of this phenomenon is related not to the axon itself but to the entire organism. Throughout the organism histological and immediate functional characters are intimately related, and any attempt to belittle the functional significance of the former indicates a failure to appreciate the complex relations of function itself. Of more direct significance are those variations of axons involving their length, their diameter and the nature of their sheaths; there is a very definite difference in the type of conduction in medullated and non-medullated axons, and in axons of different diameters. But in spite of such variations axons constitute a well-defined group in that the axon always differs radically both histologically and functionally from the other portion of the neuron, namely, the cell body with its dendrites. The cell bodies and dendrites have a higher rate of metabolism and present a greater variation in histological character than do axons, and one would expect such structures to exhibit a wide range of activity. The gray matter does exhibit a much more varied activity than do the axons, but certain investigators attribute these activities exclusively to the synapse and attribute to the cell body and dendrites pure axon or peripheral types of conduction. Later it will be shown that the high metabolic rate and varied histological character of the cell body and its dendrites are in reality associated with a wide range of local activity, and that this type of local activity differs radically from that of the axon. It should be noted that comparative measurements of cell bodies which do not take into account at least the larger dendrites may be misleading; the result is especially unfortunate when one compares the size of cell bodies of the peripheral afferent and efferent neurons, for in the former dendrites are poorly developed or absent, while in the latter a large portion of the cell body cytoplasm has grown out towards regions of excitation.

The two portions of the neuron, namely, the axon and the cell body with any extensions of its own type of cytoplasm (dendrites) which the latter may possess, may in mammals be related to the course of conduction in one of three different ways. In typical peripheral afferent neurons the cell body gives rise to a single axon which by dividing affords a continuous

axon pathway from sensory surface to central gray matter. Another type of relation is exhibited by the bipolar neurons of the vestibular ganglion in which conduction is from one axon directly through the cell body to the other axon. In the third and commonest type the cell body is related, often through dendrites, to the axon of a preceding neuron; as in the bipolar neuron conduction from axon to axon is inevitably through the cell body, but in this case the two axons belong to separate neurons and a synapse intervenes between the first axon and the succeeding cell body. Thus conduction from axon to axon may in different cases be direct, through a cell body or through a synapse and cell body. It is then evident that the pure axon conduction studied in nerve trunks should not be contrasted with merely one type of central conduction but with three, and that as conduction passes from the pure axon type (peripheral type of conduction) in one axon to the pure axon type in a second axon the process of conduction is subject to one of three modifying mechanisms. The axon conduction throughout unipolar neurons is possibly modified by the cell body and is best termed simply axon conduction as contrasted with pure axon conduction; conduction between the axons of such bipolar neurons as have two axons is intracellular conduction, while between the axons of other neurons conduction is intercellular or synaptic. Sherrington's identification of peripheral and reflex conduction with intracellular and intercellular conduction respectively is thus without basis of fact, as is the resulting conclusion that all the special features of reflex conduction are due to the synapse. It should be noted that in mammals there is a clearer division of the neuron into two distinct portions than often obtains in lower vertebrates; this is related to the high development in mammals of synaptic, polarized conduction. The synapse is a region of increased resistance separating two different types of cytoplasm which belong to different cells (neurons).

II. THE RELATION OF CELL BODY AND DENDRITES TO THE PHENOMENON OF CENTRAL FATIGUE

Are all the special features which characterize conduction within the central gray matter dependent exclusively upon the synapse? The great diversity of histological character in cell body and dendrites of different neurons is difficult to explain on the basis of relatively unmodified axon conduction within these structures. On the other hand, one is not justified in assuming that histological changes within the cell body following prolonged conduction are an indication of a specialized type of conduction, for they might be related exclusively to those metabolic activities which maintain the specific protoplasm of the entire neuron. Sherrington reaches the conclusion that the synapse is the only structure in the central gray matter capable of modifying pure axon (or peripheral) conduction, a conclusion based on

considerations presented in two successive paragraphs (Sherrington 1910, pp. 14 and 15). In the first paragraph he concludes that passage through the cell body cannot modify axon conduction since it apparently does not do so in the dorsal root ganglia; but, as has been noted, one would expect essentially axon conduction through these ganglia. He indeed admits the possibility that conduction through the ganglia may be directly from axon to axon but sees in this no contradiction to his previous assumption that the impulse passes through the cell body, regarding it as an indication of the lack of importance of the cell body for the modification of conduction in general. His error is due largely to the failure to distinguish between the different relations which may exist in different regions between cell body, axon and synapse. In the next paragraph (p. 15) he attempts to eliminate the dendrites, stating that dendrites within the gray matter are often directly continuous with axons and are themselves nerve fibers. In this connection one must distinguish between the relations within the dorsal root ganglia and those within the central gray matter. Within the dorsal root ganglia synaptic conduction is very imperfectly understood and need not be discussed. Within these ganglia there is a continuity of the two divisions of the single process of the cell body, and on the basis of direction of conduction one is at liberty to term the peripheral branch a "dendrite"; in this sense there would exist a continuity between dendrite and axon, and the peripheral branch is certainly a nerve fiber. Such a "dendrite" resembles the dendrites of the central gray matter in direction of conduction or, more exactly, in the distal location (with respect to the cell body) of the point at which the stimulus must normally act. But there is no basis for the assumption that this similarity indicates that the dendrites of the central gray matter resemble the peripheral sensory nerve fibers in all other respects or indeed in any other respect. Within the central gray matter axons may arise not directly from a cell body but from one of the larger dendrites, and when such a continuity exists the dendrite does not resemble a nerve fiber but on the contrary is (in mammals) clearly a direct extension of the cell body cytoplasm. Accordingly there is no evidence to indicate that in mammals conduction through the cell bodies and dendrites of the central gray matter is essentially of the same type as through the peripheral sensory neuron; nor is there any basis for the resulting conclusion that the modification of conduction within the central gray matter depends exclusively upon the synapse.

The relation of the cell body and dendrites to reflex conduction may be studied through histological analysis of the nervous mechanisms employed in different types of reactions. In undertaking such an analysis one must bear in mind that the histological character of a neuron corresponds to its local (intraneuronic) activity, and that while this local activity is employed in the execution of the reaction, its use is one which involves some essen-

tial detail of execution. The problem, then, is to determine how the special histological character exhibited by the cell body and dendrites of a given neuron corresponds to such functional demands as are imposed by the reaction, not upon the entire mechanism, but upon the individual neuron in question. In attempting to determine the functional significance of histological character in the cell body and dendrites, the best point of attack is the relative resistance to central fatigue.

Neither the afferent nor the efferent peripheral neurons are readily subject to direct fatigue of the individual neuron itself. Whenever a reaction exhibits early fatigue, this phenomenon must involve either one or more synapses or else the cell body or dendrites of one or more intermediate neurons. The peripheral efferent neurons possess large cell bodies with large dendrites, while the corresponding afferent neurons are usually characterized by an all axon path and in the absence of such a path their cell bodies are relatively large. But in different mechanisms the intermediate neurons differ widely in the volume of cell body and dendrites. In most exteroceptive reactions (reactions initiated by stimuli arising in the outer world) the path involves the use of intermediate neurons with small cell bodies; while in most proprioceptive reactions (reactions initiated by stimuli arising within the organism itself) it is limited to neurons with large cell bodies, the cell bodies and dendrites of the intermediate proprioceptive neurons often resembling those of the peripheral efferent to a marked degree. Whenever the mechanism of a reaction is known, it is found that those reactions which are incapable of being long sustained involve a pathway which leads through cell bodies and dendrites in which the cytoplasmic volume is small and often exceedingly small. Whereas those reactions of known mechanism which are capable of being long sustained are handled by intermediate neurons with large cell bodies and dendrites. In other words, a reaction tends to show central fatigue when it employs one or more neurons with a small cell body, and it does not tend to show fatigue when all the cell bodies employed are large. The conclusion appears inevitable that in mammals the central fatigue of reflex conduction is a property of the cell body and dendrites which is rendered effective by the resistance of the preceding synapse.

The marked histological difference between the two portions of the neuron is accordingly associated with a profound difference in the type of conduction. Moreover, concerning the differences exhibited by various types of cell bodies and dendrites, it is evident that variations in size are associated with corresponding differences in conduction, and this suggests that other histological distinctions exhibited by these structures may likewise be associated in part with corresponding differences in conduction. For it appears probable that the difference between axon (or peripheral) and reflex conduction is based in part upon the phenomenon of central fatigue with the corresponding variation of irritability; and that the special fea-

tures of reflex conduction could not be associated with any structure in which the all or none law of response obtains. The relative fatigue exhibited by large and small cell bodies determines not merely the length of time during which the reaction can persist, but it also determines the delicacy of the nervous adjustment. In the exteroceptive reception centers and in the higher correlation centers the delicacy of adjustment probably does not depend upon the interruption of the pathway so much as upon the rapidly changing condition within cell bodies and dendrites. The larger cell bodies and dendrites, due to their greater resistance to local fatigue, must respond locally with relative uniformity to similar stimuli, because these stimuli act upon structures whose irritability tends to be relatively uniform. The more labile activity of centers containing small cell bodies is to be expected, for the easily modified irritability of these small cell bodies would insure a different type of local or immediate response under slightly changed conditions of stimulation.

The size of the cell body, then, is an indication of the relative uniformity of its irritability, and as a rule this character is reinforced by other histological characters of the neuron. For instance, small cell bodies are numerous, are packed closely together, and the resulting centers have intricate connections; moreover, neurons with small cell bodies usually possess poorly developed dendrites and extensively branched axons, with the result that the impulses are diffused widely and do not tend to enter definite common paths. All of these characters reinforce the properties of the small cell body to produce a labile type of activity. On the other hand, neurons with large cell bodies are fewer in number and the architecture of such centers is simpler; as a rule, large cell bodies have extensive dendrites as compared to the relatively simple axon, characters which tend to direct the impulses into definite common paths. And in this case the associated characters reinforce the relatively uniform irritability of the large cell body to produce a more or less stereotyped activity.

III. FUNCTIONAL SIGNIFICANCE OF HISTOLOGICAL CHARACTERS IN PERIPHERAL NEURONS

But as previously noted, neurons cannot be divided into separate unrelated types, and while the local activity of neurons with very small cell bodies probably always results in diffusion, the local activity of others with somewhat larger and of those with large cell bodies may result either in diffusion or else in integration. In other words, different neuron types often exhibit with respect to histological and functional character that interrelation which one would expect from their fundamentally similar type of irritability. The peripheral afferent neurons are resistant to direct fatigue

due to a continuous axon path or else to a relatively large cell body directly continuous with both axons, and yet they are not concerned in integration but in diffusion; their local activity may be termed stable diffusion as contrasted with the stable integration of the peripheral efferent neurons and with the labile diffusion of the correlation neurons with very small cell bodies. This stable type of diffusion of the peripheral afferent neurons is absolutely essential to any highly developed central nervous system. For if these neurons were of the integrating type, the incoming impulses would come into relation with a limited portion or the gray matter and their subsequent adjustment would be greatly restricted. Whereas a labile type of activity would, through fatigue of the peripheral neuron, render impossible an almost continuous stream of incoming impulses, and due to variable irritability of the small cell body such impulses as did reach the central gray matter would not accurately represent the activity of the peripheral sense organs at any one moment, for they would vary according to the past activity of the peripheral afferent neuron.

The peripheral efferent somatic neurons exhibit great variation in the size of the cell body, but in no case is the size very small and apparently none of them is readily subject to direct fatigue. Efferent centers differ in the nature of their discharge, but it is not known how such differences are related to the size of the cell body and the corresponding variation in irritability. In these neurons larger cell bodies are apparently associated with long axons and with connections to a larger number of structures. This same relation often occurs in other portions of the nervous system in that systems which connect widely separated centers often contain neurons fewer in number but with larger cell bodies. Apparently such restriction upon the number of neurons with increase in size of cell body provides a more efficient mechanism for long-distance transmission, probably related to the more rapid conduction of their thick axons. The dendrites of peripheral efferent somatic neurons are large and extensively branched, but their character indicates more than an extensive collecting system. Their size, length and definite distribution indicate the reception of practically continuous stimuli which come always from numerous but definite sources. Thus in these neurons the functional connections of both axon and dendrites are relatively constant, as is the irritability of the large cell body. The peripheral efferent somatic neurons are employed in integration, in responding to the separate activities of neurons which are numerous but which have relatively definite functional connections; thus the activity is of a relatively stereotyped nature. The Nissl substance of these neurons is arranged in large, discrete granules, their discreteness being more characteristic than their large size. The exact significance of these discrete granules is not apparent, but such granules are always associated with the integrative capacity of a neuron.

In this section the terms somatic and visceral are used to distinguish two histological and functional classes of efferent neurons, one of which supplies skeletal muscle and the other structures of visceral function; histologically and functionally the so-called "special visceral" neurons belong to the somatic group (Malone, 1913).

The preganglionic efferent visceral neurons resemble the peripheral efferent somatic except that all their histological characters are less pronounced; the cell body is smaller and has Nissl granules which are both smaller and less discrete, while the dendrites are less developed. When peripheral efferent somatic neurons differ in size of the cell body, the small cell body appears to be essentially a small copy of the larger, and while the dendrites are usually less numerous and extensive their appearance gives the impression of a mere quantitative reduction. But in those preganglionic efferent visceral neurons with smaller cell bodies all efferent characters undergo not only a reduction in size but other changes as well. For in such neurons the outlines of cell body and dendrites are less sharp, the Nissl granules less discrete and the dendrites reduced out of proportion. The various resulting types resemble neurons in certain coordination centers, and as a matter of fact the preganglionic centers are themselves essentially coordination centers (Malone, 1923*a*). The fact that these visceral neurons differ from one another in a manner which does not apply to the peripheral efferent somatic neurons is possibly due in part to their innervation of at least three different types of structures. Why is it that these preganglionic visceral neurons, engaged as they are in extremely stereotyped reactions, appear to be less specialized in efferent histological characters than the peripheral efferent somatic neurons? Should not one expect them to exhibit even more specialized efferent characters? As far as concerns smooth muscle, this tissue has an intrinsic tone and the visceral neurons do little more than modify this tone, whereas the innervation of skeletal muscle requires a very different type of activity. The principal reason for the slightly specialized efferent histological characters of the visceral efferent system is that this system itself is primitive, with a simple architecture which necessitates an inherently automatic activity. Whereas the peripheral efferent somatic neurons, as their histological characters indicate, are in reality much more highly specialized, for they maintain the stability of the reaction through the integration of a wealth of stimuli from the most diverse sources. In these two classes of efferent neurons one can compare the histological characters associated with different degrees of integrative capacity. It appears certain that in all integrating types of mammalian neurons the volume of the cell body and dendrites is related to the relative volume of the stimuli received rather than to their variety in source; whereas the capacity to integrate activities of various sources appears to depend upon the presence

of numerous dendrites extending to distinct regions and upon the presence of large, discrete Nissl granules. In neurons of high integrative capacity each of the numerous dendrites represents an outgrowth of a large portion of the cell body directly towards a distant but definite point of intense excitation, and accordingly such dendrites are massive and possess a long and definite longitudinal axis.

IV. THE RELATION OF THE AFFERENT AND EFFERENT SYSTEMS TO CORRELATION AND COORDINATION, AND THE RELATION BETWEEN HISTOLOGICAL AND FUNCTIONAL CHARACTERS IN VARIOUS TYPES OF CORRELATING AND COORDINATING NEURONS

Afferent and efferent are terms which do not necessarily imply different types of local activity within individual neurons but which relate merely to the intake and output of the central nervous system or of any of its centers. Neurons which are afferent with respect to a center supply it with the impulses whose adjustment constitutes the activity of that center, while neurons which are efferent with respect to the center carry away impulses which represent its completed activity. One is justified in applying these terms to two systems, one consisting of neurons which are usually ascending and are concerned in planning the general type of reaction, while the other system is composed of neurons which are usually descending and which execute the general plan of the reaction. Landacre (1922) has designated the planning activity of the afferent centers "correlation" and the executive activity of the efferent centers "coordination." What Malone (1923*a* and *b*) unfortunately has termed "efferent correlation" is simply coordination. As pointed out by Strong (1925, pp. 637 to 639), the activity of the afferent system in general involves differentiation and diffusion, while that of the efferent involves fusion and integration. But these two systems are by no means sharply separated through radically different characters in all their neurons, for in each system certain neurons possess characters which resemble more or less those usually exhibited by the neurons of the other system. The direction of conduction is always by more or less indirect paths from afferent to efferent periphery, and both ascending and descending conduction may occur in either system. The predominance of ascending conduction in afferent neurons and of descending in efferent is entirely due to the oral location of the dominating centers; the fundamental indifference of these two systems to direction of conduction may be seen in the branching of the entering posterior root fibers and of the medial longitudinal fasciculus. Moreover, due to the fact that connections between the two systems are established at various levels, many afferent centers are partly concerned in coordination and contain cell bodies of the general efferent type (Malone,

1923*b*). On the other hand, correlating neurons occur in coordinating centers where the execution of the response requires delicate adjustment. Finally in both systems neurons occur which are neither purely correlating nor purely coordinating. Each system, then, may in part exhibit the direction of conduction and type of adjustment characteristic of the other system, and both employ various types of adjustment intermediate between pure correlation and pure coordination.

Exteroceptive reception centers are concerned principally in that labile type of adjustment termed by Landacre "correlation," although they contain certain cell bodies of the coordinating type. And the proprioceptive centers, with the exception of the posterior column nuclei, consist exclusively of various types of coordinating neurons (Malone, 1923*b*). Nevertheless it is advisable to consider all primary reception centers afferent and to consider their function as the determination of the general plan of the reaction, but the plan in different reactions may be determined either by a process of correlation or of coordination. Coordinating neurons may differ in type as do the peripheral efferent somatic neurons, exhibiting merely those differences to be expected in different sizes of cell body; or they may exhibit various deviations from this general type, much as do the preganglionic visceral neurons. Among coordinating neurons which closely resemble the peripheral efferent somatic are those of the lateral vestibular nucleus and certain neurons whose cell bodies are located in the reticular formation of the brain stem, the type extending through the subthalamic region into the globus pallidus (Malone, 1913). The entire cerebellar mechanism is coordinating with the exception of the correlating neurons with small cell bodies located in the cerebellar cortex (Malone, 1923*b*). The cell bodies and dendrites of the lateral vestibular nucleus are strictly of the coordinating type, while those of the lateral reticular nucleus are less so, and the coordinating type of the dentate nucleus is less marked than that of the roof nuclei. The Purkinje cells have large cell bodies but the Nissl granules do not stand out very sharply, characters related to the presence of a single massive dendrite whose numerous branches supply one continuous region instead of many distinct regions. In the inferior olive and in the nuclei pontis the cell bodies are smaller than those of the Purkinje cells but have much the same type of Nissl granules; the internal structure is again related probably to a rather simple type of integration, since apparently the sources of stimulation, while numerous, are local and not distinct. The cell bodies of the nucleus dorsalis are large, and have rather well-developed dendrites and a few large Nissl granules; but their form is atypical, due to the atypical dendrites. One should note that in so far as the form of a cell body is of functional significance, it is determined essentially by the number, size and form of the neuron's processes, and that modifications due to compression by fiber masses are without functional significance.

The relation of the nucleus dorsalis to the peripheral efferent neurons is by no means direct, involving as it does the coordinating cerebellum, and yet its cell bodies resemble to a limited extent those of typical coordinating centers. The nucleus dorsalis gives rise to long axons ascending to a higher coordinating center; it is instructive to compare these neurons to those whose cell bodies are situated in correlating centers and whose axons form long ascending paths to higher correlating centers of mammals. The cell bodies of such neurons are never very small, although they vary in size; their dendrites constitute a diffusely distributed localized collecting apparatus, while their internal structure does not exhibit large, discrete Nissl bodies. Their cell bodies occur in relation to numerous small cell bodies of typical correlating neurons, and since in these long ascending paths there is a reduction in the number of neurons as compared with the center of origin, it is evident that a fusion or integration has occurred. As a matter of fact, the activity of all neurons involves both integration and diffusion, but the functional relation of these long ascending neurons of the correlating centers is very different from that of integrating neurons of the coordinating centers. For in the former the impulse is diffused throughout the center and there is no mechanism such as occurs in coordinating centers to effect a constant converging discharge upon a few larger cell bodies. These long ascending neurons to higher correlating centers are apparently concerned in a more or less stable type of activity which differs from that of the peripheral afferent neurons in that it is less purely diffusive and from that of the nucleus dorsalis in that the integration is superimposed upon a more labile and diffuse type of activity. Just as the peripheral afferent neurons deliver to the exteroceptive reception centers a dependable indication of the activity of the sense organs, so these long ascending neurons must deliver to higher correlating centers a dependable indication of the labile and diffuse activity of the lower centers, in each case the axon termination being sufficiently diffuse to permit the receiving center to correlate these impulses with those from other sources. It is essential to realize that while the activity of the entire correlation center, due to connections to different regions of the nervous system, always involves differentiation and diffusion, this character does not necessarily apply to the individual neuron. For in addition to the locally diffusing type of neuron such centers may contain long ascending neurons and also true coordinating neurons. From their localized nature all centers must effect a spreading of impulses, but the dispersion is wider from correlating centers and is superimposed upon a local diffusion of the individual correlating neurons with labile activity.

Correlation due to its labile activity is usually confined to the first or planning portion of the reaction, but in at least one instance it may follow coordination; this subsequent correlation is exhibited by the small neurons of the cerebellar cortex. Proprioceptive reactions may occur at one of several

levels; such reactions may involve the level of the vestibular nucleus, the internal cerebellar nuclei, the Purkinje cells and finally the small correlating cortical neurons. The latter through their labile diffusive activity evidently supplement the general coordinating activity of the cerebellar apparatus and thus permit of a more delicately adjusted execution. It is probable that correlation may follow cerebellar coordination also in the red nucleus and thalamus and in regions associated with these centers.

V. CONCLUSION

One may conclude that the size of the cell body is related to the volume of its activity and to its tendency to maintain a uniform irritability. One may also conclude that the form of a neuron is an indication of its collecting and diffusing capacity; and that its internal structure is related to the capacity to integrate impulses from distinct sources, this capacity involving also the presence of numerous long dendrites to distinct regions. It is evident that correlation may involve the stable diffusion of the peripheral afferent neurons, the labile diffusion of the typical correlating neurons with small cell bodies and, finally, the activity of the long ascending neurons. The latter effect a correlation between centers and involve an integration of the diffuse activity of the lower center, together with a fairly diffuse axon termination in the higher center; this type of correlation certainly approaches the less characteristic types of coordination. It is also evident that coordination is characterized by an integration and that coordinating neurons differ widely in histological character according to the volume of their activity and the variety in source of the stimuli received. Correlation and coordination are types of activity which differ in the ease with which the adjustment can be modified, and while as a rule the earlier adjustments in a reaction are more easily modified than are the later, frequent exceptions occur.

The individual constituents of the gray matter are inaccessible to direct functional study but they are accessible to histological analysis, and it is possible to relate such analyses in neurons of different mechanisms to the functional characters of mechanisms employed in different types of reactions. While the normal activities of the organism permit only general conclusions as to the local function of the individual neuron, they furnish the most dependable of all physiological data. It is only by being content for the present with very general conclusions that one can advance beyond the recognition of types of neurons to investigate the relations of different neuron types and the broad functional significance of individual histological characters.

VI. BIBLIOGRAPHY

Coghill, G. E. 1924. Correlated anatomical and physiological studies of the growth of the nervous system in amphibia iii. *J. Comp. Neurol.*, **37**, 37.

Herrick, C. J. 1924. *Neurological foundations of animal behavior.* N. Y.: Henry Holt.

Landacre, F. L. 1922. Quoted in Herrick's *Introduction to neurology.* Phila.: W. B. Saunders Co.

Malone, E. F. 1913. Recognition of members of the somatic motor chain of nerve cells by means of a fundamental type of cell structure, and the distribution of such cells in certain regions of the mammalian brain. *Anat. Rec.*, **7**, 67.

—— 1923*a*. The cell structure of the superior olive in man. *J. Comp. Neurol.*, **35**, 205.

—— 1923*b*. Efferent characteristics of reception centers. *Science*, **57**, 449.

Sherrington, C. S. 1910. *The integrative action of the nervous system.* New Haven: Yale University Press.

Strong, O. S. 1925. In *Bailey's textbook of histology.* N. Y.: Wm. Wood & Co.

Section XXIX

THE SYMPATHETIC NERVE CELLS

CONTENTS

SECTION XXIX

	PAGE
I. MORPHOLOGY	1009
II. INTERNAL STRUCTURE	1010
1. Neurofibrils	1010
2. Chromidial substance	1011
3. Nucleus	1014
4. Nucleus-plasma ratio	1015
III. AXON	1016
IV. DENDRITES	1018
V. CELL CAPSULES	1019
VI. IS THERE A STRUCTURAL BASIS FOR A CLASSIFICATION OF SYMPATHETIC NEURONS INTO FUNCTIONAL TYPES?	1019
VII. PATHOLOGY	1023
1. Pigmentation	1023
2. Changes in Chromidial Substance	1024
3. Inflammatory and degenerative processes	1025
VIII. BIBLIOGRAPHY	1026

Section XXIX

THE SYMPATHETIC NERVE CELLS

ALBERT KUNTZ

DISREGARDING the physiological classification of the autonomic nervous system into a sympathetic and a parasympathetic division, all the neurons in the body which lie outside the cerebrospinal nervous system and are functionally connected with the latter through the visceral components of the cerebrospinal nerves may be regarded as sympathetic nerve cells. In general, these cells are aggregated in ganglia, many of which contain large numbers, others relatively few neurons. Sympathetic ganglia are widely distributed in the body and sustain definite relationships to peripheral nerves. The ganglia of the sympathetic trunks are intimately connected with the spinal nerves, through the communicating rami. The cranial sympathetic ganglia are similarly connected with the cranial nerves. The prevertebral sympathetic ganglia are connected with the spinal nerves by visceral components which pass through the sympathetic trunks. Sympathetic ganglia lying farther peripherally, including those in the walls of visceral organs, are related to the central nervous system both through the vagus and spinal nerves. The visceral efferent components of the cerebrospinal nerves terminate in synaptic relationship to sympathetic neurons. Synaptic relationships of sympathetic neurons with visceral afferent components of the cerebrospinal nerves are unknown.

I. MORPHOLOGY

In the majority of the sympathetic ganglia the neurons are compactly aggregated. They are usually arranged in groups or columns separated somewhat by the framework of supporting tissue and a complex of nerve fibers, including the visceral components of the cerebrospinal nerves which either terminate in the ganglion or pass through it, and an intercellular plexus made up of the axons and dendritic processes of the sympathetic ganglion cells.

In mammals, the great majority of the sympathetic neurons are multipolar cells, having a single axon and a number of dendritic processes varying from several to perhaps twenty or more. These cells vary greatly in form and size. The cell body may be well rounded, somewhat angular, definitely elongated or irregular in outline. In certain locations, e.g., in the myenteric plexus, many of the sympathetic cells are definitely flattened. In man, according to Spiegel and Adolf (1922), the greatest diameter of the cell body of the majority of the sympathetic neurons falls within a range of 35μ to 45μ,

while the maximum diameter of the cell bodies of the smaller sympathetic neurons is only 20μ to 25μ. They also observed occasional large spindle-shaped sympathetic neurons whose long axis measured 50μ to 60μ.

In birds, the sympathetic neurons are mainly of the multipolar type, similar in form and structure to those of mammals. However, the ciliary ganglion in birds seems to contain only unipolar nerve cells (Von Lenhossek, 1911; Carpenter, 1911). In reptiles, sympathetic neurons of the multipolar type probably preponderate. In the turtle, Huber (1899) found multipolar neurons in all the sympathetic ganglia examined. However, both bipolar and unipolar sympathetic neurons also occur. Von Lenhossek (1911) found only small unipolar neurons in the ciliary ganglia in this class of vertebrates. In amphibia, neurons of the unipolar type preponderate in the ganglia of the sympathetic trunks, although multipolar neurons also occur in these ganglia (Smirnow, 1900). In certain of the peripheral sympathetic ganglia, e.g., those in the enteric plexuses, in amphibia, the neurons are all of the multipolar type (Huber, 1899; Nemiloff, 1901). In fishes, although bipolar and unipolar sympathetic neurons occur, the majority of the sympathetic cells are multipolar.

II. INTERNAL STRUCTURE

1. *Neurofibrils:*

Neurofibrils are commonly present throughout the cytoplasm of the cell body as well as in the axon and the dendritic processes. They vary greatly in abundance and configuration. In preparations in which the neurofibrils are well stained and abundant in the majority of the cells, certain cells may be found in which neurofibrils appear only in the cell processes and in the peripheral parts of the cell body. In such cells the neurofibrils also appear to be more delicate than in the cells in which the neurofibrillar structure is abundant. In optical section the neurofibrils appear, in many of the cells, to run, singly or in small bundles, through the cell body in various directions (Fig. 482B.). In some cells the neurofibrils exhibit a more complex configuration (Fig. 482A). In general, those in the deeper portions of the cytoplasm seem to be arranged with reference to the nucleus; those in the more superficial parts, with reference to the cell processes and the periphery of the cell body. Not infrequently these two configurations seem to be quite distinct, although not independent of each other. Thus Cajal (1905) described a perinuclear and a peripheral neurofibrillar network. In favorable preparations, neurofibrils running parallel with each other may be observed in the cell processes throughout nearly their entire length.

Our present knowledge of the neurofibrillar substance in sympathetic cells depends to a large extent on isolated fragmentary observations recorded by a large number of investigators. This is due in part to the difficulty in

obtaining preparations favorable for the study of this substance in sympathetic cells. On the other hand, relatively few investigators have made neurofibrils a special object of study. Among the latter should be mentioned Laignel-Lavastine (1906) and Michailow (1908). The majority of the recorded observations bear primarily on (1) the relation of the neurofibrils to each other, and (2) the location of the various kinds of neurofibrillar structure with reference to the parts of the cell.

In mammals, according to Cajal (1905) and Laignel-Lavastine (1906), the neurofibrillar substance in the sympathetic neurons comprises a fairly compact perinuclear network and a less compact peripheral network which are not connected with each other by interlacing or anastomosing fibrils. Michailow (1908), who attempted to classify sympathetic neurons in mammals according to their neurofibrillar structure, recognized a perinuclear and a peripheral neurofibrillar network in certain sympathetic cells, but pointed out that in some sympathetic cells in which the neurofibrillar substance constitutes a real network, perinuclear and peripheral portions of this network cannot be differentiated. In some of these cells, the perinuclear zone appears to be quite devoid of neurofibrils. Without regard to the details of Michailow's classification, which comprises some sixteen types of neurofibrillar structure in sympathetic neurons, it should be pointed out that he recognizes, in many of the sympathetic cells, a neurofibrillar structure in which the fibrils neither give rise to branches nor anastomose with each other, but permeate the entire cell body and its processes. While individual neurofibrils may be traced from the dendritic processes or from the axon into the cell body, where they ramify according to some particular type of configuration, individual neurofibrils can rarely be traced from one cell process, through the cell body, and into another cell process.

Regarding the finer structure of the neurofibrils in sympathetic neurons, we have no definite knowledge at present. These cell components probably are not to be regarded as homogeneous structures. However, none of the methods at present available seems adequate for the study of their constituent elements (see p. 971). Even casual observations reveal a wide range of variation in the caliber and configuration of these fibrils. Detailed studies like those of Laignel-Lavastine and Michailow strongly suggest that these variations are significant, although no important conclusions can be drawn from our present knowledge regarding them. Michailow (1908) believes that the large caliber of the neurofibrils in some sympathetic neurons represents hypertrophy, and may be of pathological significance.

2. Chromidial substance (Nissl substance, tigroid bodies, chromophile substance, etc.):

The chromidial substance does not exhibit as wide a range of variation in the size and configuration of the chromidial bodies in sympathetic as in

cerebrospinal nerve cells (see p. 999). In general, the chromidial bodies are larger and less closely aggregated in the sympathetic than in the afferent, but smaller and more closely aggregated than in the efferent cerebrospinal nerve cells. The chromidial substance is distributed, in sympathetic neurons, according to three common modes; viz., aggregated in the periph-

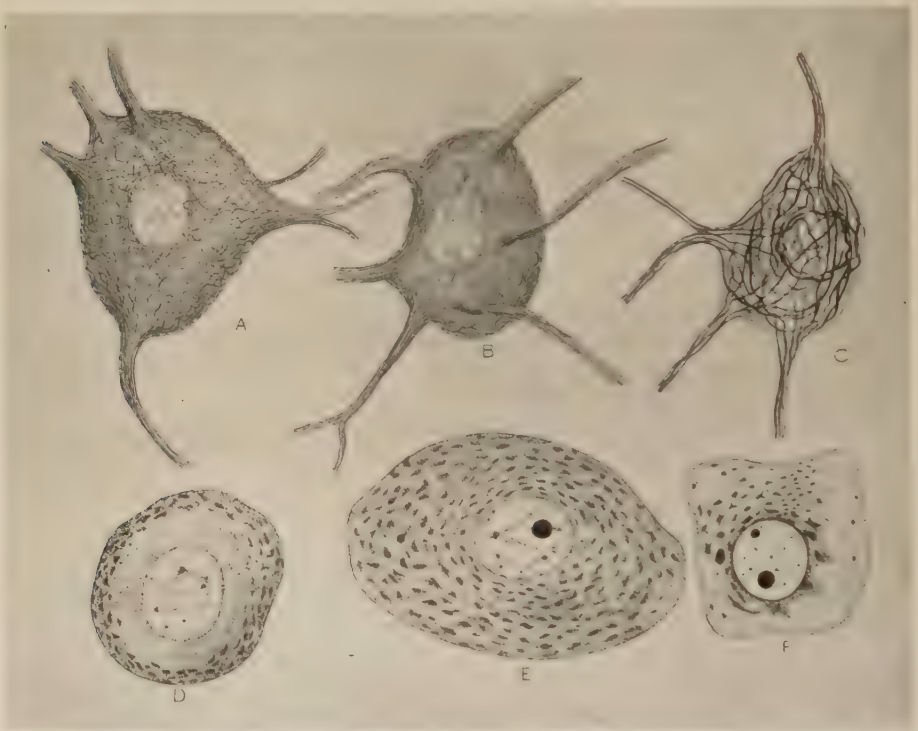


FIG. 482.—A and B, camera lucida drawings from a pyridine silver preparation of a ganglion of the sympathetic trunk of the dog, showing distribution of neurofibrils in sympathetic cells (Spencer, oc. 10x, obj. 1.8).

C, sympathetic ganglion cell showing thickened neurofibrils. (After Michailow.)

D and E, camera lucida drawings from sections of the superior cervical sympathetic ganglion of the dog, showing distribution of the chromidial substance in sympathetic cells (Spencer, oc. 10x, obj. 1.8).

F, sympathetic ganglion cell showing perinuclear distribution of the chromidial substance, superior cervical ganglion of the rat. (After Ping.)

eral zone (Fig. 482F), aggregated in the perinuclear zone (Fig. 482F), or distributed quite uniformly throughout the cytoplasm of the cell body and the proximal portions of the dendrites (Fig. 482D). Intergradations between fairly uniform distribution and aggregation in the peripheral or perinuclear zones are not uncommon. The chromidial bodies are relatively small in

the perinuclear zone and gradually increase in size toward the periphery. Not uncommonly, regardless of the particular mode of distribution, the chromidial bodies exhibit a net-like configuration.

In man, according to Spiegel and Adolf (1922), all these modes of distribution of the chromidial substance are common in the larger sympathetic neurons (35 to 40μ). In the smaller ones, the chromidial bodies are usually fairly uniformly distributed throughout the cytoplasm. They are also relatively fewer and more delicate than in the larger cells. Carpenter and Conel (1914) described essentially the same distribution and configuration of the chromidial bodies in the sympathetic neurons in rodents.

The chromidial substance arises relatively early in the sympathetic cells. In man it is fairly abundant, even before birth. According to Marinesco (1906), the chromidial granules appear earliest near the periphery of the cell and gradually migrate toward the nucleus. Vas (1892) described a fairly abundant chromidial network in the sympathetic cells in a full term fetus. In the newborn the chromidial substance in the sympathetic cells exhibits practically the same range of variation in structure and distribution as in the mature condition (Spiegel and Adolf, 1922).

In the larger nerve cells in the superior cervical sympathetic ganglia in the albino rat, Ping (1921) was able to recognize progressive changes in the quantity of the chromidial substance and in the character and distribution of the chromidial bodies. During the first twenty days of postnatal life, the chromidial bodies are relatively small and fairly evenly distributed throughout the cytoplasm. During the latter part of this period, the beginning of aggregation of the chromidial bodies becomes apparent in many of the cells. From the twentieth to the sixtieth day of postnatal life the chromidial bodies in the cells studied are larger and stain more intensely than during the preceding period. In the majority of these cells the chromidial substance is aggregated either in the peripheral or the perinuclear zone. Both these modes of distribution of the chromidial substance occur also following this period. However, during adult life many of the sympathetic neurons included in the group under consideration exhibit a fairly uniform distribution of chromidial bodies throughout the cytoplasm.

In the sympathetic nerve cells, as in the neurons in the cerebrospinal nervous system (see p. 971) the chromidial substance appears to be affected by cell activity and fatigue. Following stimulation of the cervical sympathetic ganglia for fifteen minutes, Vas (1892) observed enlargement of the cells and migration of the chromidial bodies toward the periphery. Lambert (1893) observed similar migration of the chromidial bodies toward the periphery, following stimulation, but no changes in the size of the cells. Mann (1894) observed enlargement during activity and shrinking both of the nucleus and the cell body in fatigue. He also observed enlargement of the chromidial bodies during activity and reduction in the quantity of

the chromidial substance during prolonged stimulation. Lugaro (1895) also observed an initial enlargement both of the cell body and the nucleus during activity. According to his observations the cell body reaches its maximum enlargement in five minutes; the nucleus, in thirty minutes. After this both cell body and nucleus decrease in size rapidly during the first few hours, and then more slowly. He also observed an increase in the quantity of the chromidial substance during the early phases of activity, and a diminution in quantity and a more diffuse distribution of this substance during the later phases of cell activity and in fatigue. According to Sternschein (1923), section of the preganglionic fibers to the superior cervical sympathetic ganglion (cat, rabbit) results in shrinking of the sympathetic cell body and characteristic changes in the chromidial substance. The chromidial bodies become larger, assume a somewhat fibrous appearance, and become more compactly aggregated. They also react more intensely to basic stains, and net-like configurations become more apparent. Following faradic stimulation of this ganglion, he observed swelling of the cell bodies and a degree of central chromatolysis.

3. *Nucleus:*

In the sympathetic neurons, as in the neurons in the cerebrospinal nervous system (see p. 967), the nucleus is relatively large, rounded or oval in outline, and contains relatively little stainable material except one or more well-defined nucleoli. In favorable preparations the nuclear membrane and the reticular structure in the nucleus are distinctly visible. The nucleus is usually centrally located, but may not infrequently be found in an eccentric position.

The great majority of the sympathetic neurons are uninuclear cells; however, binuclear sympathetic neurons are not uncommon, especially in rodents. Occasional multinuclear sympathetic neurons have also been observed. According to Müller (1909), binuclear sympathetic neurons occur only rarely in man. On the other hand, Spiegel and Adolf (1920) maintain that binuclear sympathetic neurons are not uncommon, especially in young persons. They also observed multinuclear sympathetic cells in the newborn. According to their observations, binuclear sympathetic neurons gradually decrease in number with advancing age. In some of their preparations obtained from middle-aged persons, they could still find binuclear cells in nearly every section. In preparations obtained from aged persons, they could find binuclear sympathetic cells only very rarely.

The observation that binuclear sympathetic cells are relatively common in the young and decrease in number as age advances suggests, according to Spiegel and Adolf (1922), that these cells retain the capacity to undergo division without further nuclear changes. Like Laignel-Lavastine (1906), they regard them as reserve cells. That such cells actually undergo division

is also suggested by the occasional occurrence of two sympathetic neurons in a common cell capsule.

The occurrence of binuclear neurons in great abundance in the ganglia of the sympathetic trunks and prevertebral plexuses was noted by many investigators in a wide variety of rodents. However, Apolant (1896) called attention to the fact that, in these animals, binuclear neurons occur but rarely in the sympathetic ganglia associated with the cranial nerves. Carpenter and Conel (1914) corroborated this observation and pointed out further that, in the rodents studied by them (rabbit, guinea pig, muskrat porcupine), they were also unable to find binuclear neurons in the enteric plexuses. They suggested that the common occurrence of binuclear neurons in the thoracolumbar sympathetic ganglia and their absence in the enteric plexuses and the other sympathetic ganglia associated with cranial nerves might constitute a morphological basis which would justify Langley's classification of the autonomic nervous system into a sympathetic and a parasympathetic division. However, a comparable distribution of binuclear and uninuclear sympathetic neurons has not been observed in other groups of mammals.

4. *Nucleus-plasma ratio:*

By careful measurements Spiegel and Adolf (1922) found that the nuclei of the larger sympathetic neurons in man have a diameter of 12μ to 13μ, while those of the smaller ones have a diameter of 8μ to 12μ. The sympathetic cell bodies, excluding those which are definitely elongated, vary from 20μ to 45μ in diameter. They also observed that in young children the nuclei of the sympathetic cells are nearly uniform in size, although some of these cells contain very little cytoplasm. Obviously the nuclei of the smaller sympathetic cells are larger in proportion to the volume of the cytoplasm than are the nuclei of the larger cells. Apparently, as the cell grows, the nucleus does not keep pace with the cytoplasm. According to Ping's (1921) studies on the growth of the largest nerve cells in the superior cervical sympathetic ganglia in rats, the nucleus-plasma ratio of these cells is 1 : 4 in the albino and 1 : 5 in the Norway rat at birth, and 1 : 12 in both varieties at maturity.

The nucleus-plasma ratio of mature sympathetic neurons of approximately the same size is probably fairly constant. However, when all the neurons in a given sympathetic ganglion in the mature condition are considered, the nucleus-plasma ratio shows a wide range of variation. Heidenhain (1907) expressed the opinion that nerve cells constitute an exception to Hertwig's law of the constancy of the nucleus-plasma ratio by reason of the fact that neuroblasts have the capacity to produce short or long axons, according to their location or the requirements of the tissues innervated by them. However, the inconstancy in the nucleus-plasma ratio is especially

striking in sympathetic neurons, even though the cell processes are disregarded.

In view of the inconstancy of the nucleus-plasma ratio in nerve cells, Heidenhain believes that the chromidial substance in the cytoplasm, which is histochemically related to the chromatin in the nucleus (p. 968) is present in relatively greater quantity in the larger than in the smaller cells, especially if the larger cells have long axons. Therefore, since the chromidial substance in the cytoplasm is functionally related to the chromatin in the nucleus, he believes the nucleus-plasma ratio may still be regarded as constant.

III. AXON

The axon of a sympathetic neuron may arise directly from the cell body or from the proximal portion of a dendritic process. The implantation cone, or axon hillock, is usually less conspicuous in the sympathetic cells than in the motor cells in the central nervous system. As in the latter, it is free from chromidial substance. Neurofibrils may be traced from the cell body through the implantation cone into the axon, where they lie closely aggregated and parallel with each other. The axons of sympathetic neurons are, in general, fibers of relatively small caliber and unmyelinated. However, many of them are clothed in a very thin myelin sheath throughout at least a portion of their length. In the absence of a myelin sheath the thin nucleated neurilemma is closely applied to the axon. Even in the presence of a thin myelin sheath, the neurilemma still lies so close to the axon that nodes or incisures cannot usually be recognized. Such fibers are quite commonly regarded as unmyelinated. In mammals sympathetic axons are probably rarely myelinated throughout their entire length. The myelinated axons acquire their myelin sheaths at unequal distances from their origin. The ratio of myelinated to unmyelinated sympathetic nerve fibers varies in different regions of the body, in types of different species and in types of the different classes of vertebrates. However, the data at present available do not justify any definite conclusions regarding the ratio of myelinated to unmyelinated sympathetic fibers in any vertebrate group. In mammals, the sympathetic axons which join the spinal nerves through the communicating rami are probably myelinated in greater proportion than those distributed to the visceral organs, although myelinated axons arising from cells in the prevertebral sympathetic ganglia have been observed. The axons arising from the cells in the ciliary ganglia are quite generally myelinated (Huber, 1913). In birds, according to Langley (1896), the axons of all the neurons in the ganglia of the sympathetic trunks are myelinated; consequently, true gray communicating rami do not occur in birds. Medullated sympathetic fibers have frequently been described in amphibia. Most of the accounts bearing on this problem in reptiles and fishes refer only to

unmyelinated sympathetic nerve fibers. The data available indicate no functional differences in sympathetic nerve fibers which are correlated with the absence or presence of a myelin sheath.

Since the great majority of the sympathetic cells are postganglionic neurons in visceral efferent chains, their axons terminate in relation to the tissue elements innervated by them, viz., muscle and gland cells. Individual axons cannot usually be traced from their origin to their termination; however, occasional observations are recorded in which a sympathetic axon was traced without interruption throughout its entire course.

In smooth muscle, the sympathetic axons commonly form plexuses surrounding the fasciculi. From these plexuses fine fibers, either singly or in small bundles, pass into the muscle fasciculi where in general they run parallel to the muscle fibers and give off delicate branches which terminate on the muscle cells. These terminal branches have been described by many observers and in various parts of the body as ending on the smooth muscle cells in one or two terminal nodules. Such terminal nodules are regarded by Hofmann (190-) as artefacts. He maintains that there exists in reality an end plexus composed of fibrils which form loops which come into relation with the muscle cells, but the fibrils go on without interruption. Michailow (1908) also maintains that there exists an end plexus. However, he also described free terminations which, when stained by appropriate methods, show ring-like and net-like fibrillar formations. He contends that there are free fiber terminations, but that the neurofibrils are everywhere continuous.

In the cardiac muscle, the sympathetic axons also form plexuses surrounding the fasciculi, from which fine fibers, either singly or in small bundles, penetrate the fasciculi and in general run parallel to the muscle fibers. These fibers give off delicate branches which terminate on the cardiac muscle fibers in one, two or several nodular enlargements.

Terminations of sympathetic axons in relation to gland cells have been described in various types of glands. According to most observers, these axons form a plexus situated external to the membrana propria (epilamellar plexus of Arnstein), from which fibers may be traced through the membrana propria without further branching or plexus formation to their terminations on gland cells. These terminations are always delicate, but exhibit a wide range of complexity and configuration.

Since the publication of Boeke's experimental anatomical studies (1913), in which he first pointed out clearly that certain fine unmyelinated fibers which had previously been described as terminating on skeletal muscle cells are of sympathetic origin, so much evidence has accumulated in favor of this view that a distribution of sympathetic axons to skeletal muscle cells may be regarded as an anatomical fact. These fibers, according to Boeke, terminate in delicate end rings or loops and end nets which resemble very closely the terminations of sympathetic axons on smooth muscle cells. They

are hypolemmal in position and rest on granular sole plates through which the fine reticular periterminal network in the sarcoplasm comes into relation with the neurofibrils. The majority of the investigators who have described the terminations of sympathetic axons in skeletal muscles in general corroborate Boeke's description. In the panniculus carnosis of the hedgehog, Garven (1925) recently described, in addition to the end rings and end nets referred to above, terminations of certain fine unmyelinated fibers, which he also regards as sympathetic, which resemble the usual somatic motor end plates in miniature. He also described certain of the unmyelinated fibers in this muscle as anastomosing with each other to form a plexus lying between the muscle fasciculi, from which delicate fibers arise which terminate on the muscle fibers in the usual end rings or end loops.

IV. DENDRITES

The dendrites of sympathetic neurons exhibit a wide range of variation both in number and in their general morphological characters. They are essentially extensions of the cell body, usually broad at the base and tapering distally. Commonly they give rise to branches and frequently exhibit varicosities and other irregularities. In favorable preparations, neurofibrils may be traced well into their terminal branches. Chromidial bodies, like those in the peripheral portions of the cell body, also occur in the broader proximal portions of the dendrites. Short dendrites usually terminate within the cell capsule. The longer dendrites usually penetrate the cell capsule and ramify in the intercellular plexus or extend beyond the limits of the ganglion in the fiber bundles associated with it.

Dogiel (1896), in his early work, recognized sympathetic neurons of several types, depending primarily on the character and distribution of their dendrites. In his first type he included neurons with many short branching dendrites. These he designated as motor sympathetic neurons. In his second type he included neurons with relatively few long dendrites which branch only sparingly for some distance from the cell body, but give rise to several fine terminal branches which extend beyond the boundaries of the respective ganglion. These he believed were sensory sympathetic neurons. In still another type he included certain neurons with long, slender dendrites which do not extend beyond the boundaries of the respective ganglion, but ramify in the intercellular dendritic plexus. Cajal (1905), basing his classification on the character and distribution of their dendrites, also described sympathetic neurons of three types. His first type included neurons with long, branching dendrites which radiate from the cell body in all directions. His second type included neurons with relatively long, frequently branching dendrites which arise from one side of the cell body and interlace with similar dendrites of a neighboring neuron to form

a glomerulus. Michailow (1911), basing his classification on the character, distribution and manner of termination of their dendrites, described nine distinct types of sympathetic neurons. Müller (1924) recognized the three types of sympathetic neurons described by Cajal, but pointed out that there are many sympathetic neurons which do not conform to any of these types. All these attempts to classify sympathetic neurons according to the character and distribution of their dendrites only emphasize the variability of these processes, and add little or nothing to our knowledge of the functional relationships of the sympathetic cells.

V. CELL CAPSULES

In all the larger sympathetic ganglia the cell bodies of the neurons are enclosed in delicate cell capsules. These cell capsules are made up of a layer of small endothelial cells with elongated, flattened nuclei and a delicate, structureless membrane in contact with which the endothelial cells lie (Fig. 483). The neurilemma of the axon is continuous with the cell capsule. As already pointed out, short dendrites commonly terminate within the cell capsule, while the longer ones usually penetrate it and ramify in the intercellular plexus. In immediate proximity with the cell capsule are slit-like lymph spaces which are connected with the other lymph spaces in the ganglion (Key and Retzius, 1876). This relationship suggests an important rôle of the endothelial cells lining the capsule in the metabolic interchange of materials as well as in degenerative processes involving the sympathetic cells. In certain of the peripheral sympathetic ganglia, especially in the enteric plexuses, many of the neurons probably have no cell capsule.

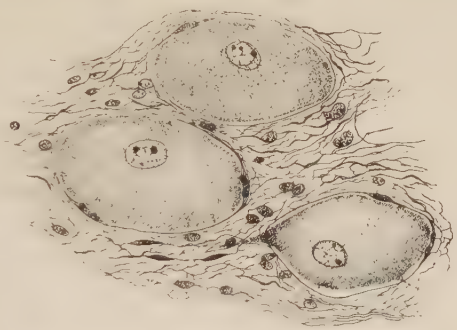


FIG. 483. Camera lucida drawing from a section of the superior cervical sympathetic ganglion of the dog, showing sympathetic cell capsules. (Spencer, oc. 10x, obj. 1.8.)

VI. IS THERE A STRUCTURAL BASIS FOR A CLASSIFICATION OF SYMPATHETIC NEURONS INTO FUNCTIONAL TYPES?

The great majority of the sympathetic neurons, as already indicated, are postganglionic neurons in visceral efferent chains. They are, therefore, essentially efferent in function. However, not a little physiological evidence has been brought forward which strongly suggests the existence of afferent

sympathetic neurons, especially in the peripheral sympathetic plexuses. Attempts have also been made to associate differences in function with structural differences in the sympathetic nerve cells.

As we have mentioned, Dogiel (1896) advanced the theory that the sympathetic neurons included in his first morphological type are motor, while those included in his second morphological type are sensory in function. However, the work of later investigators indicates clearly that sympathetic neurons of the types which Dogiel regarded as motor and sensory respectively only represent the extremes in a wide range of morphological variation.

Studies of the internal structure of sympathetic neurons, especially the character and configuration of the chromidial bodies, also reveal no significant differences with which differences in function can be correlated (Carpenter and Conel, 1914). Such characteristic differences in the form, size and configuration of the chromidial bodies as are exhibited by the afferent and efferent neurons in the cerebrospinal nervous system (Jacobsohn, 1910; Malone, 1913) do not occur in sympathetic neurons. Neither can differences in function be correlated with differences in the structure and configuration of the neurofibrils in sympathetic nerve cells (Michailow, 1909).

As certain writers have suggested (Carpenter and Conel, 1914; Müller, 1924, and others), morphological differences in sympathetic cells might conceivably be correlated with the particular organs or tissues innervated by them. Even such a theory is beset with difficulties by reason of the very wide range of morphological variation exhibited by sympathetic nerve cells. Nevertheless, sympathetic neurons approximating the first type described by Cajal, i.e., neurons with relatively long, branching dendrites which radiate from the cell body in all directions, preponderate in the ganglia of the sympathetic trunks and prevertebral plexuses (Müller, 1909). Neurons with short intracapsular dendrites preponderate in the ciliary, otic and sphenopalatine ganglia, while in the submaxillary ganglion the majority of the neurons are characterized by relatively long, slender dendrites which do not penetrate the cell capsule, but form an intracapsular network around the cell body (Müller and Dahl, 1910). The neurons in the enteric plexuses show a wide range of variation (Fig. 484), but differ characteristically from those in other parts of the sympathetic system. Many of the neurons in the myenteric plexus are characterized by numerous short dendrites which terminate in relation to the gastrointestinal musculature. Very commonly these cells lie in immediate proximity with the muscle and are not enclosed in cell capsules (Fig. 484A, D). According to Müller (1920), cells of this character occur in considerable numbers in the myenteric plexus in man. Cells of the same character and relationship also occur in the myenteric plexus in the dog (Fig. 484D). Neurons with relatively long dendrites which do not terminate in relation to the musculature but ramify

in the intercellular plexus are also common. These cells are usually somewhat flattened and show the widest distribution of their dendrites in the tangential plane (Fig. 484B, C). The neurons in the submucous plexus are less variable and more compactly aggregated in the ganglia than those in the myenteric plexus. Their dendrites are commonly broad at the base and taper rapidly. They may very commonly be traced for some distance

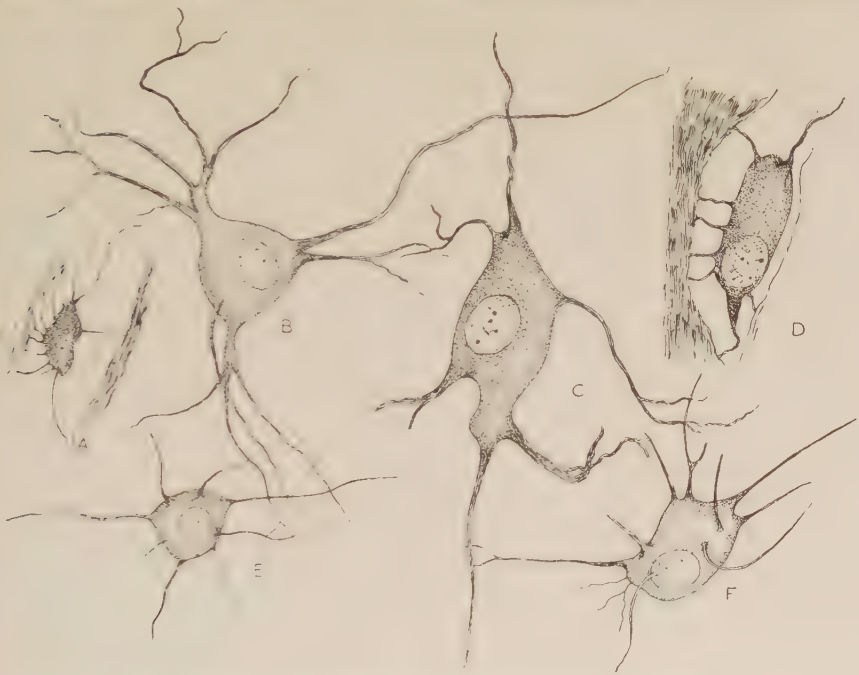


FIG. 484.—Sympathetic ganglion cells in enteric plexuses.

A, myenteric neuron (human intestine) with short dendrites which terminate in relation to musculature. (After Müller.)

B, C, D, camera lucida drawings of myenteric neurons, from pyridine silver preparations of the small intestine of the dog. (Spencer, oc. 10x, obj. 1.8.)

E and F, camera lucida drawings of neurons in the submucous plexus, from pyridine silver preparations of the intestine of the dog. (Spencer, oc. 10x, obj. 1.8.)

beyond the limits of the respective ganglion. Figure 484 (E and F) illustrates neurons of a type which is very common in the submucous plexus in the dog.

Although morphological characters alone afford no certain criteria for a functional classification of sympathetic neurons, these characters, in conjunction with the relationships of the neurons to each other and with their orientation, especially in the peripheral sympathetic plexuses, with respect to the surrounding tissues, may not inconceivably afford an index to differences in function.

Terminations of dendrites of sympathetic neurons in pericellular networks on the cell bodies of other sympathetic neurons have been described in the superior cervical sympathetic ganglia (Cajal, 1905; L. R. Müller, 1909). However, the functional significance of such relationships and the relationships of sympathetic neurons brought about through the intercellular dendritic plexuses is not known. Certain investigators, notably E. Müller (1921), regard the enteric plexuses as consisting in part of nerve nets composed of cells whose processes anastomose freely. Neither Kuntz (1922) nor Johnson (1925) was able to find anastomoses between neurons in the enteric plexuses. Furthermore, as Johnson has shown very clearly, the cells which E. Müller described as constituting a syncytial nerve net in the intestinal wall are probably not nerve cells, but connective tissue elements. If anastomoses between sympathetic nerve cells occur at all in mammals, they are relatively uncommon. Neither have we any knowledge regarding the possible functional significance of such relationships.

Huber (1913) described the terminations of fine varicose nerve fibers, presumably of sympathetic origin, on the dendrites of sympathetic neurons. Kuntz (1922) also described terminations of sympathetic fibers on the cell bodies of sympathetic neurons. In methylene blue preparations of the intestine of the cat, a process of a sympathetic cell which showed an intense staining reaction could in some instances be traced without interruption to its termination in a pericellular network on the lightly stained cell body of another neuron in the same ganglion. Although these pericellular networks resemble very closely the pericellular terminations of preganglionic fibers on sympathetic neurons and were interpreted as synapses, they might still be regarded as pericellular terminations of dendrites. However, in other instances, fibers which enter a ganglion in the myenteric plexus through a ramus which connects this ganglion with a ganglion in the submucous plexus could be traced to their terminations in similar pericellular networks on the cell bodies of lightly stained sympathetic neurons. These terminations must be regarded as true synapses. Furthermore, inasmuch as the fibers involved run from the submucous into the myenteric plexus, they cannot reasonably be interpreted as preganglionic visceral efferents, but are, doubtless, the axons of sympathetic neurons. Identical terminations of fibers which enter a given ganglion through a ramus which connects it with a neighboring ganglion in the same plexus were observed on the lightly stained cell bodies of sympathetic neurons, both in the myenteric and submucous plexuses. Some of these, especially in the myenteric plexus, probably represents synapses of preganglionic fibers with sympathetic neurons. In all probability many of them represent synapses involving two sympathetic neurons. Two sympathetic neurons which sustain a synaptic relationship with each other cannot, according to current teaching, be

regarded as terminal links in a visceral efferent chain. They cannot reasonably be interpreted except as components of a local reflex arc.

In this connection it is interesting to note that Müller (1924) regards it as misleading to ascribe a motor function to those neurons in the myenteric plexus whose short dendrites terminate in relation to muscle fibers. Their relationships seem to meet the requirements of neurons which are adapted to transmit impulses arising in the musculature. Müller also suggests that certain of the myenteric neurons which are characterized by long dendrites may mediate afferent impulses which arise in the gastrointestinal mucosa, although he could not demonstrate terminations of nerve fibers in the digestive epithelium. Terminations of delicate nerve fibers in the digestive epithelium were described by Sacussew (1897) in fishes, and by Kuntz (1922) in mammals. In favorable preparations fiber bundles, including dendrites of ganglion cells in the submucous plexus, may also be traced through the muscularis mucosae and into the intestinal villi (Kuntz). These findings, in conjunction with the synaptic relationships between enteric neurons described above, strongly support the theory that sympathetic neurons which are essentially afferent in function exist, at least in the enteric plexuses.

VII. PATHOLOGY

Our knowledge regarding pathological changes in sympathetic neurons is still very meager. There are at present no exact criteria available by which transitions from normal physiological to initial pathological processes may be recognized. Furthermore, there is a relatively wide range of normal physiological and morphological variation. Doubtless, progressive changes commonly observed in sympathetic neurons with advancing age must be be regarded as physiological.

1. *Pigmentation:*

Moderate pigmentation of sympathetic neurons is a common phenomenon in man after middle age. In certain diseases, including senile atrophy and cachexia, many of the sympathetic neurons become heavily pigmented and also exhibit other evidences of degenerative changes. Although moderate pigmentation of these cells must probably be regarded as physiological, exaggerated pigmentation is probably always a pathological phenomenon.

The beginning of pigmentation of the sympathetic cells in man, according to Pilcz (1895), may usually be observed in the second year of life. Vas (1892) described yellow pigment in the sympathetic cells in one case in a full term fetus. Lubinoff (1874) found some pigmented sympathetic cells in fetuses of five and seven months. Spiegel and Adolf (1922) found traces of yellow pigment in the sympathetic cells in the newborn. They

also pointed out that pigmentation is less common in the neurons in the cranial sympathetic ganglia and the enteric plexuses than in those in the ganglia of the sympathetic trunks and the prevertebral plexuses. Marked pigmentation of the sympathetic cells in any part of the body is not commonly observed until the age of forty.

The pigment in the sympathetic cells is of two kinds; viz., a yellow lipoid pigment which reacts to fat stains and is at least partially soluble in alcohol, ether and other fat solvents, and a darker, more stable pigment which does not react to stains and is highly insoluble (Pilcz, 1895; Obersteiner, 1903; Marinesco, 1906, and others). The dark pigment is similar in appearance to the pigment in the substantia nigra. However, the latter pigment is not associated with yellow pigment. In the sympathetic cells yellow pigment usually precedes the darker pigment. The data available also seem to indicate that in the sympathetic cells the dark pigment arises from the yellow pigment (Spiegel and Adolf, 1922). As age advances the yellow pigment commonly disappears, leaving only dark pigment in the majority of the pigmented sympathetic cells.

Pigmented sympathetic cells exhibit a wide range of variation in the distribution of the pigment. Not uncommonly pigment occurs only in a narrow peripheral zone. In some instances it is aggregated in an angle of the cell body or at the base of the axon. Occasionally it occurs in a cap-shaped mass at the periphery of the nucleus. In certain elongated cells in which the greater part of the cytoplasm is aggregated toward the two poles, a mass of pigment may appear toward either end of the cell body. In persons over sixty years of age, in cachexia and in arsenic poisoning pigment may be distributed quite uniformly throughout the cytoplasm of the sympathetic cells and become so dense that the nucleus is obscured and the cell appears as a mass of dark pigment (Spiegel and Adolf, 1922). Heavy pigmentation of the sympathetic cells is also fairly constant accompaniment of atrophy of the sympathetic ganglia (Graupner, 1898; Laignel-Lavastine, 1906, and others).

2. *Changes in the chromidial substance:*

In certain pathological conditions, e.g., arsenic poisoning and poliomyelitis, the chromidial bodies in the sympathetic cells break up and the chromidial substance apparently goes into solution (see p. 971). Such chromatolysis may involve the majority of the sympathetic cells. In general, chromatolysis of a few sympathetic cells need not be regarded as pathological. During senility and cachexia many of the larger chromidial bodies, especially in the perinuclear zone, break up. In cells which are not heavily pigmented, the chromidial substance in the perinuclear zone may appear as chromidial dust. Fragmentation of the chromidial bodies may continue until the chromidial substance throughout the cell body seems to

be in solution. In Nissl preparations, such cells exhibit a homogeneous blue color with possibly a few heterogeneous patches (Vas, 1892; Laignel-Lavastine, 1906; Spiegel and Adolf, 1922). In cases of death due to burns, the sympathetic cells exhibit clumping of the chromidial substance (Spiegel and Adolf, 1922).

3. *Inflammatory and degenerative processes:*

Inflammation of the sympathetic ganglia (sympathoganglionitis) is an accompaniment of many infective diseases. Not uncommonly the character of the inflammatory process in the sympathetic ganglia is typical of the infection (Abrikossoff, 1923). In the initial stage of inflammation the endothelial cells lining the sympathetic cell capsules proliferate and the capsular walls become materially thickened. Many cells become separated from the endothelium and lie free in the lumen of the capsule where they assume the rôle of phagocytes and take part in the destruction of the sympathetic cell

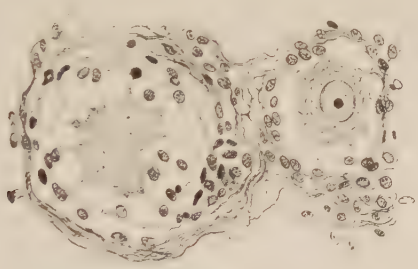


FIG. 485.—Proliferation of the endothelial cells of the capsules and necrosis of the sympathetic ganglion cells in a septic condition. (After Stämmler.)

(Fig. 485). These changes are usually accompanied by infiltration by other cells (lymphocytes and leucocytes) which also take part in the phagocytic process. In this manner neuronophagia may continue until the destruction of the sympathetic cell is completed (Spiegel and Adolf, 1922). After the inflammatory process has subsided, nothing remains in the lumen of the thickened capsule in such cases but amorphic material, fragmented cells and colloidal masses (Stämmler, 1923). Chronic inflammatory processes are not uncommonly accompanied by thickening of the cell capsule without neuronophagia. Such thickening is brought about by splitting of the outer membrane, proliferation of the endothelial cells and rearrangement of these cells in concentric layers.

Initial degenerative changes in sympathetic cells are not uncommonly manifested by blanching, both of the cytoplasm and the nucleus. The cytoplasm assumes a homogeneous appearance. The nucleus which is normally oval in outline becomes spherical. The chromatin becomes diffused and reacts poorly to basic stains. The nucleolus becomes enlarged. Later the

nucleus shrinks and reacts intensely to basic stains but shows no characteristic nuclear structure. It may assume a position at the periphery of the cell or be extruded. Such cells, as indicated above, commonly become heavily pigmented and exhibit chromatolysis in varying degrees. In silver preparations, the neurofibrillar substance also exhibits marked changes. The network becomes coarser and the neurofibrils are materially thickened. Later the neurofibrils become fragmented and the cell body assumes a vacuolated and fenestrated appearance. Finally the cell body may undergo shrinkage and withdraw from the thickened walls of the capsule, leaving the intervening space filled with lymph.

The significance of pathological changes in sympathetic nerve cells is as yet little understood. However, such changes must be recognized as of far-reaching importance in the clinical course of many diseases by reason of functional disturbances, especially of the circulatory and glandular systems and the gastrointestinal musculature, which are directly referable to impairment of the sympathetic innervation. The pathology of the sympathetic nervous system is a fertile field for further investigation.

VIII. BIBLIOGRAPHY

Abrikossoff, A. I. 1923. Die pathologische Anatomie der sympathischen Ganglien. *Virchow's Archiv,* **240,** 281.

Apolant, H. 1896. Ueber die sympathischen Ganglienzellen der Nager. *Arch. f. mikr. Anat.,* **47,** 461.

Azoulay, L. 1904*a*. Les neurofibrilles d'après la méthode et les travaux de S. Ramon y Cajal. *La presse médicale,* 165, 537, 585, 635.

——— 1904*b*. Les neurofibrilles dans les cellules nerveuses situées autour du tube digestif de la sangsue. *C. rend. Soc. de Biol.,* **56,** 465.

Berkley, H. J. 1894. On complex nerve-terminations and ganglion cells in the muscular tissue of the heart-ventricle. *Anat. Anz.,* **9,** 33.

Boeke, J. 1913. Die doppelte (motorische und sympathische) efferente Innervation der quergestreiften Muskelfasern. *Anat. Anz.,* **44,** 343.

Brauer, L. 1895. Beitrag zur Lehre von den anatomischen Veränderungen des Nervensystems beim Morbus Addisonii. *Deutsch, Ztschr. f. Nervenh.,* **7,** 417.

Cajal, S. R. 1905. Las céllulas del gran sympathico del hombre adulto. *Trab. del Lab. de invest. Biol. de la Universidad de Madrid,* **5.**

——— 1911. *Histologie du système nerveux. a. Maloine:* Paris.

Calligaris, G. 1908. Beitrag zum Studium der zellen des Locus coeruleus, usw *Monatschr. f. Psych. u. Neur.,* **24,** 335.

Carpenter, F. W. 1906. The ciliary ganglion of birds. *Folia Neuro-Biol.,* **5,** 704.

——— 1911. The ciliary ganglion of birds. *Ibid.,* **5.**

——— 1912. On the histology of the cranial autonomic ganglia of the sheep. *J. Comp. Neur.,* **22,** 447.

Carpenter, F. W., and Conel, J. L. 1914. A study of ganglion cells in the sympathetic nervous system, with special reference to intrinsic sensory neurons. *Ibid.,* **24,** 269.

Dogiel, A. S. 1895*a*. Zur Frage über den feineren Bau des sympathischen Nervensystems bei den Säugethieren. *Arch. f. Mikr. Anat.*, **46**, 305.

——— 1895*b*. Zur Frage über die Ganglien der Darmgeflechte bei den Säugethieren. *Anat. Anz.* **10**, 517.

——— 1896. Zwei Arten sympathischer Nervenzellen. *Ibid.*, **11**, 679.

——— 1898. Zur Frage über den feineren Bau der Herzganglien des Menschen und der Säugethieren. *Arch. f. Mikr. Anat.* **53**, 237.

——— 1899. Ueber den Bau der Ganglien in den Geflechten des Darmes und der Gallenblase des Menschen und der Säugethiere. *Arch. f. Anat. u. Phys., Anat. Abth.*, 130.

Eve, F. C. 1896. Sympathetic nerve-cells and their basophil constituent in prolonged activity and repose. *J. Physiol.*, **20**, 334.

Garven, H. S. D. 1925. The nerve-endings in the panniculus carnosus of the hedgehog, with special reference to the sympathetic innervation of striated muscle. *Brain*, **48**, 380.

Graupner, R. 1898. Beiträge zur normalen und pathologischen Anatomie des sympathischen Nervensystems, *Beitr., z. path. Anat. u. z. allg. Path.* Ziegler's, **24**, 255.

Heidenhain, M. 1907. *Plasma und Zelle.* Jena.

Hertwig, R. 1903. Ueber Korrelation von Zell- und Kerngrösse. *Biol. Zentralbl.* **23**, 49, 108.

Hofmann, F. B. 190–. Histologische Untersuchungen über die Innervation der glatten und der ihr verwandten Muskulatur der Wirbelthiere und Mollusken. *Arch. f. mikr. Anat.*, **70**, 361.

Holtzmann, H. 1896. Untersuchungen über Ciliarganglion und Ciliarnerven. *Morph. Arbeit.*, **6**, 114.

Huber, G. C. 189–. Lectures on the sympathetic nervous system. *J. Comp. Neur.*, **7**, 73.

——— 1899. A contribution to the minute anatomy of the sympathetic ganglia of the different classes of vertebrates. *J. Morph.*, **16**, 27.

——— 1913. The morphology of the sympathetic system. *Folia Neuro.-Biol.*, **7**, 616.

Jacobsohn, L. 1910. Struktur und Funktion der Nervenzellen. *Neur. Centralb.*, **29**, 1074.

Johnson, S. E. 1925. Experimental degeneration of the extrinsic nerves of the small intestine in relation to the structure of the myenteric plexus. *J. Comp. Neur.*, **38**, 299.

Juschtschenko, J. A. 189–. Zur Frage über den Bau der sympathischen Knoten. *Arch. f. mikr. Anat.*, **49**, 585.

Key, A. and Retzius, G. 1876. Anatomie des Nervensystems und des Bindegewebes. Stockholm.

Klein, C. 1904. *Sympathische Ganglienzellen der Säugethiere.* Dissert. Rostock.

Kölliker, A. 1894. Ueber die feinere Anatomie und die physiologische Bedeutung des sympathischen Nervensystems. *Verhandl. Ges. Deutsch. Naturf. u. Arz.*, 66, Theil 1, 97.

Kuntz, A. 1922. On the occurrence of reflex arcs in the myenteric and submucous plexuses. *Anat. Record*, **24**, 193.

Laignel-Lavastine, M. 1903. *Recherches sur le plexus solaire*, Thèse.

——— 1906*a*. Imprégnation argentique des neurofibrilles de l'homme. *C. rend. Soc. de Biol.*, **61**, 297.

——— 1906*b*. Imprégnation argentique des neurofibrilles du cobaye, du lapin et du chien. *Ibid.*, **61**, 364.

——— 1908. Anatomie pathologique de plexus solaire des tuberculeux. *Rev. de Méd.*, **28**, 515.

Lambert, M. 1893. Notes sur les modifications produites par l'excitation electrique dans les cellules nerveuses des ganglions sympathiques. *C. rend. Soc. de Biol.*, **9**, 879.

Langley, J. N. 1896. Observations on the medullated fibers of the sympathetic and chiefly on those of the gray rami communicantes. *J. Physiol.*, **20**, 55.

v. Lenhossek, M. 1910. Ueber das Ganglion ciliare. *Verhandl. d. Anat. Ges. 24. Versamml., Brüssel*, 137.

——— 1911*a*. Das Ganglion ciliare der Vögel. *Arch. f. mikr. Anat. u. Entwickl.*, **76**, 745.

——— 1911*b*. Das Ciliarganglion der Reptilien. *Anat. Anz.*, **40**, 74.

Lubinoff, A. 1874. Beiträge zur Histologie und pathologische Anatomie des sympathischen Nervensystems. *Virchow's Archiv*, **61**, 145.

Lugaro, E. 1895. Sur les modifications des cellules nerveuses dans divers états functionnels. *Arch. Ital. de Biol.*, **24**, 258.

Malone, E. F. 1913. Recognition of members of the somatic motor chain of nerve cells by means of a fundamental type of cell structure, and the distribution of such cells in certain regions of the mammalian brain. *Anat. Record*, **7**, 67.

Marina, A. 1899. Das Neuron des Ganglion ciliare und die zentra der Pupillen bewegungen. *Deutsch. Zeitschr. f. Nervenheilk.*, **14**, 356.

Mann, G. 1894. Histological changes induced in sympathetic, motor and sensory nerve-cells by functional activity. *J. Phys. and Anat.*, **29**, 100.

Marinesco, G. 1904. Nouvelles recherches sur les neurofibrilles. *Rev. Neur.*, 15.

——— 1906. Quelques recherches sur la morphologie normale et pathologique des cellules, ganglions spinaux et sympathiques de l'homme. *Névraxe*, **8**, 7.

Michailow, S. 1908*a*. Mikroskopische Struktur der Ganglien des Plexus solaris und andere Ganglien des Grenzstranges des Nervus sympathicus. *Anat. Anz.*, **33**, 581.

——— 1908*b*. Die feinere Struktur der sympathischen Ganglien der Harnblase bei den Säugethieren. *Arch. f. mikr. Anat.*, **62**, 554.

— 1908*c*. Die Neurofibrillen der sympathischen Ganglienzellen bei Säugethieren. *Folia Neuro-Biol.*, **1**, 637.

— 1910. Der Bau der zentralen sympathischen Ganglien. *Internat. Monatschr. f. Anat. u. Phys.*, **28**, 26.

——— 1911. Der Bau der Zentralen sympathetischen Ganglien. *Internat. Monatschr. f. Anat. u. Physiol.*, **28**, 26.

Michel, J. 1894. Über die feinere Anatomie des Ganglion ciliare. *Trans. 8th Internat. Opbthal. Congr.*, Edinburgh, 195.

Mogilnizcky, B. 1923. Die Veränderungen der sympathischen Ganglien bei Infektionskrankheiten. *Virchow's Archiv*, **241**, 298.

Morse, W. 1917. Histopathology of the autonomic nervous system in certain somatic and organic nervous diseases. *J. Nerv. and Ment. Dis.*, **45**.

Müller, E. 1921. Ueber das Darmnervensystem. *Upsala Läkareförenings förhandlingar, Ny folgd.*, **26**, 658.

Müller, L. R. 1909. Studien über die Anatomie und Histologie des sympathischen Grenzstranges, insbesondere seine Beziehungen zu dem spinalen Nervensystem. *Verhandl. d. Kongr. f. inn. Med.*, **26**, 658.

——— 1920. *Das vegetative Nervensystem*. Berlin.

——— 1924. *Die Lebensnerven*. Berlin.

Müller, L. R., and Dahl, W. 1910. Die Beteiligung des sympathischen Nervensystems an der Kopfinnervation. *Deutsch. Arch. f. klin. Med.*, **99**, 48.

Müller, L. R., and Glaser, W. 1913. Ueber die Innervation der Gefässe. *Deutsch. Zeitsch. f. Nervenh*, **46**, 325.

Nemiloff. 1901. Zur Frage der Nerven des Darmkanals bei den Amphibien. *Travana de la Sociêtê des naturalistes de St. Petersbourg*, **32**.

Obersteiner, H. 1903. Ueber das hellgelbe Pigment in den Nervenzellen. *Obersteiner's Arb.*, **10**, 245.

Pilcz, A. 1895. Beitrag zur Lehre der Pigmententwickelung in den Nervenzellen. *Arb. a. d. neur. Inst. d. Univ. Wien*, **3**, 123.

Ping, C. 1921*a*. On the growth of the largest nerve-cells in the superior cervical sympathetic ganglion of the albino rat—from birth to maturity. *J. Comp. Neur.*, **33**, 281.

——— 1921*b*. On the growth of the largest nerve-cells in the superior cervical sympathetic ganglion in the Norway Rat. *Ibid.*, **33**, 313.

Retzius, G. 1889. Zur Kenntniss der Ganglienzellen des Sympathicus. *Verhandl. d. biol. Ver. in Stockholm*, **2**, 16.

——— 1892. Ueber den Typus der sympathischen Ganglienzellen der höheren Wirbelthiere. *Biol. Untersuch.*, N. F. **3**, 57.

Rumianzeff. 1908. *Material zur Frage der pathologisch-anatomischen Veränderungen der autonomen Nervenzellen des Herzens bei Diphtherie.* Dissert. St. Petersburg.

Sacussew. 1897. Ueber die Nervenendigungen im Verdauungskanal der Fische. *Travaux de la Soc. de Nat. de St. Petersburg.*

Sala, L. 1893. Sur la fine anatomie des ganglions sympathique. *Arch. Ital. de Biol.*, **18**, 439.

——— 1910. Sulla fina struttura del ganglio ciliare. *Mem. del. R. Instit. Lombardo sci. e lett.*, **21**, 133.

Smirnow, A. 1890. Die Struktur der Nervenzellen im Sympathicus der Amphibien. *Arch. f. mikr. Anat.*, **35**, 407.

——— 1900. Zur Kenntniss der Morphologie der sympathischen Ganglienzellen beim Frosche. *Anat. Hefte*, **14**, 409.

Spiegel, E. A. 1921. Zur Morphologie der peripherischen Ganglien. *Anat. Anz.*, **54**, 331.

Spiegel, A. E., and Adolf, M. 1922. Die Ganglien des Grenzstranges. *Arb. a. d. neur. Instit. Wien*, **23**, 67.

Spielmeyer, W. 1922. *Histopathologie des Nervensystems.* Berlin.

Stämmler, M. 1923. Zur Pathologie des sympathischen Nervensystems, im besonderen über seine Bedeutung für die Entstehung der Arteriosklerose. *Beitr. z. path. Anat. u. z. allg. Pathol.*, **71**, 388.

Sternschein, E. 1922. Das Ganglion cervicale supremum nach prae- und postganglionärer Durchschneidung. *Arb. a. d. neur. Instit. Wien*, **23**, 155.

Van Gehuchten, A. 1892. Les cellules nerveuses du sympathique chez quelques mammifères et chez l'homme. *La Cellule*, **8**, 85.

Vas, F. 1892. Studien über den Bau des Chromatins in der sympathischen Ganglienzelle. *Arch. f. mikr. Anat.*, **40**, 375.

White, H. 1887, 1889. On the histology and functions of the mammalian superior cervical ganglion. *J. Physiol.*, **8**, 66; **10**, 341.

Section XXX

NEUROGLIA AND MICROGLIA
THE INTERSTITIAL TISSUE OF THE CENTRAL NERVOUS SYSTEM

CONTENTS

Section XXX

PAGE

I. Neuroglia Morphology 1034
1. Astrocytes 1034
Special types of astrocytes—Independence of astrocytes.
2. Oligodendroglia 1041
Interfascicular glia-perineuronal satellites.
3. Neuroglia contacts. 1043
Astrocyte and blood vessel—Astrocyte and pia—Astrocyte and neuron.
II. Development of Neuroglia 1044
1. Cytogenesis of astrocytes 1044
2. Cytogenesis of oligoglia. 1048
III. Overgrowth and Degeneration of Neuroglia. 1052
1. Degenerative changes. 1052
Acute—Chronic.
2. Hyperplastic change 1055
Gliosis of astrocytes—Satellitosis of oligodendroglia.
IV. Microglia, or Mesoglia of Hortega 1055
1. Morphology. 1056
2. Types 1056
V. Abnormal Forms of Mesoglia 1057
1. Rod cells 1057
2. Compound granular corpuscles. 1057
3. Origin of abnormal forms. 1058
VI. Mesodermal Origin of Microglia 1058
1. First appearance. 1058
2. "Fountains" 1059
3. Immigration. 1061
4. Morphological evidence. 1062
5. Biological evidence. 1063
VII. Function of Interstitial Cells 1064
1. Astrocytes 1064
2. Oligodendroglia 1064
3. Mesoglia 1065
VIII. Bibliography 1065

Section XXX

NEUROGLIA AND MICROGLIA
THE INTERSTITIAL TISSUE OF THE CENTRAL NERVOUS SYSTEM

WILDER PENFIELD

THE interstitial tissue of the central nervous system (Robertson, 1900*b*) includes all the non-nervous cells with the exception of the vascular tree and meninges. In no other organ of the body is there such great diversity of cell function as in the brain and cord. Such a functional differentiation is of course associated with complex physicochemical differences in cell structure. This has made necessary a wide variety of selective staining methods, "the keys which unlock . . . passage to scientific treasure" (Weigert).

Virchow (1846) was apparently the first to distinguish a ground substance in the nervous system and he came to recognize neuroglia cells and fibers. This ground substance was taken to be a sort of connective tissue until after the chrome silver method of Golgi (1886) had provided a better means of study. There followed what Huber (1903) called the Golgi period during which time neuroglia cells were usually considered as units provided with individual expansions and protoplasm.

This method was succeeded by the widespread use of the staining procedures of Weigert (1895) and of Mallory. As a result of the employment of these procedures, particularly the former, the concept of the neuroglia cell as an entity was largely given up; the fibers were considered to be *intercellular* structures divorced from cell protoplasm and a glial syncytium was described (Held, 1903).

Cajal (1913*a* and 1913*b*) next provided a new and strikingly selective method for neuroglia (gold chloride-sublimate). This method showed the astrocyte with greater completeness, demonstrating it as a unit, independent of syncytial connections. In a similar way a few years earlier, this scientist had provided a solution to the rather chaotic discussion of the so-called nervous reticulum by demonstrating the independence of neurons and their freedom from such connections (Barker, 1899). Cajal also now separated off from neuroglia that large group of small non-nervous cells, which were unstained or incompletely stained by all the neuroglia methods then in use. He called these cells the "third element" in the central nervous system.

Del Rio-Hortega (1919, 1921*b*) made the next great advance when he discovered that he could stain this "third element" selectively with silver carbonate. He found the third element of Cajal to be made up of two groups

of cells: (1) Oligodendroglia which includes the numerous "interfascicular glia" of the white matter and the larger part of the perineuronal satellites of the gray matter; and (2) Microglia which is morphologically, biologically and developmentally different from neuroglia. Oligodendroglia he included under the heading of neuroglia; while microglia (or mesoglia) he proposed as the true third element.

For the purposes of this description neuroglia and microglia will be taken up separately.

I. NEUROGLIA MORPHOLOGY

1. *Astrocytes (spider cells, Deiters' cells, common neuroglia, macroglia):*

By astrocytes is meant the classical neuroglia which Kölliker (1893) and Andriezen (1893*a*) subdivided into two types, protoplasmic cells and fiber cells. These cells have been called astrocytes because of their stellate shape, the term neuroglia referring to the rôle they were supposed to play, i.e., gluing the nervous elements together. Strong (1925) has called these cells astroglia and grouped them with oligodendroglia under the main heading of neuroglia.

Astrocytes are divided into (1) *fibrous* and (2) *protoplasmic*, according to the presence or absence of fibers in the cell cytoplasm. Normally astrocytes are all protoplasmic in the gray matter of the brain and spinal cord, while in the white matter and subpial or plexiform layer of the cerebrum the astrocytes are fiber formers. As Spielmeyer (1922) has pointed out, however, this is not always strictly true, because fibrous neuroglia may be seen also in the thalamus. In the description of the finer structure of astrocytes which follows much is borrowed from Cajal's monograph (1913*b*).

The *protoplasmic astrocyte* is provided with expansions which pass out in all directions and branch with the greatest frequency (AS.2 in Figs. 486 and 487). The *fibrous astrocyte* expansions are much straighter, less numerous and longer (AS.1 in Figs. 486 and 487). Both types are attached to a small blood vessel by one or more perivascular feet (Fig. 487).

The astrocyte *nucleus* is irregularly oval. It is larger than oligodendroglia or microglia nuclei (Fig. 488) and has chromatin which is scattered and small in amount. The absence of nucleolus serves to distinguish it from neuron nuclei (p. 967).

The *cytoplasm* of astrocytes is a little granular when stained by gold. It has a finely reticular structure as pointed out by Del Rio-Hortega (1916*a*). When stained with acid fuchsin the cytoplasm is seen to contain rather fine regular fuchsinophile *granules* (Alzheimer, 1910). These granules are also normally present in nerve cells as shown by this stain. Under pathological conditions, however, the neuroglia granules increase in size and may form lipoid cysts.

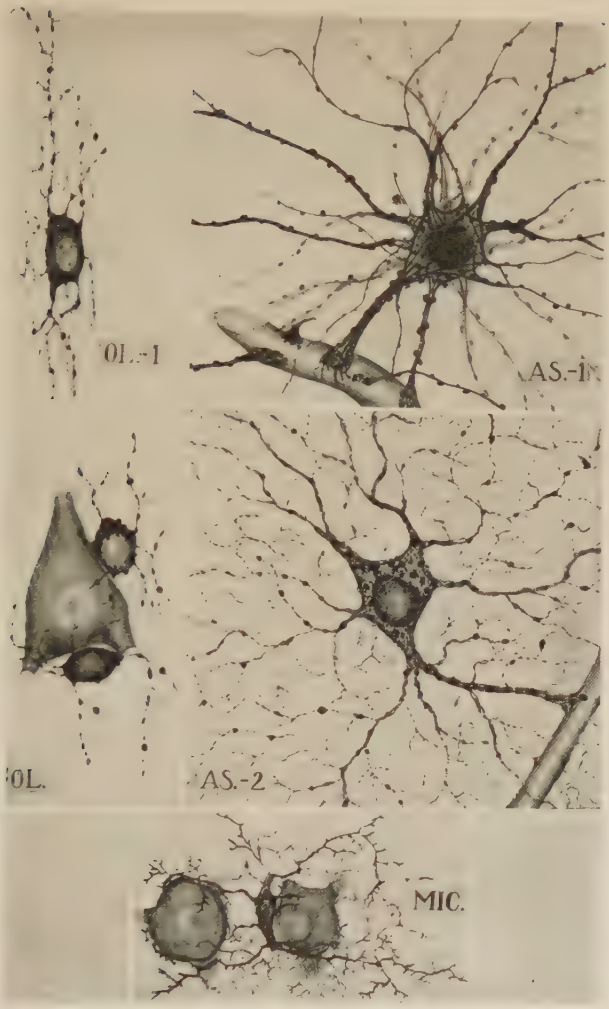


FIG. 486.—The interstitial cells of the central nervous system: drawings. Lettering same as in Fig. 487. Gliosomes are seen in all four types of neuroglia cells. The oligodendroglia is drawn from man, having a somewhat more voluminous cytoplasm than that seen in laboratory animals. Microglia (MIC.) and oligodendroglia (OL.-2) of gray matter are shown in relation to nerve cells. (Modified after Penfield and Cone, 1926*b*.)

The *centrosome* is placed at the base of the largest vascular expansion. When astrocytes are arranged in pairs or fours, the centrosomes are placed

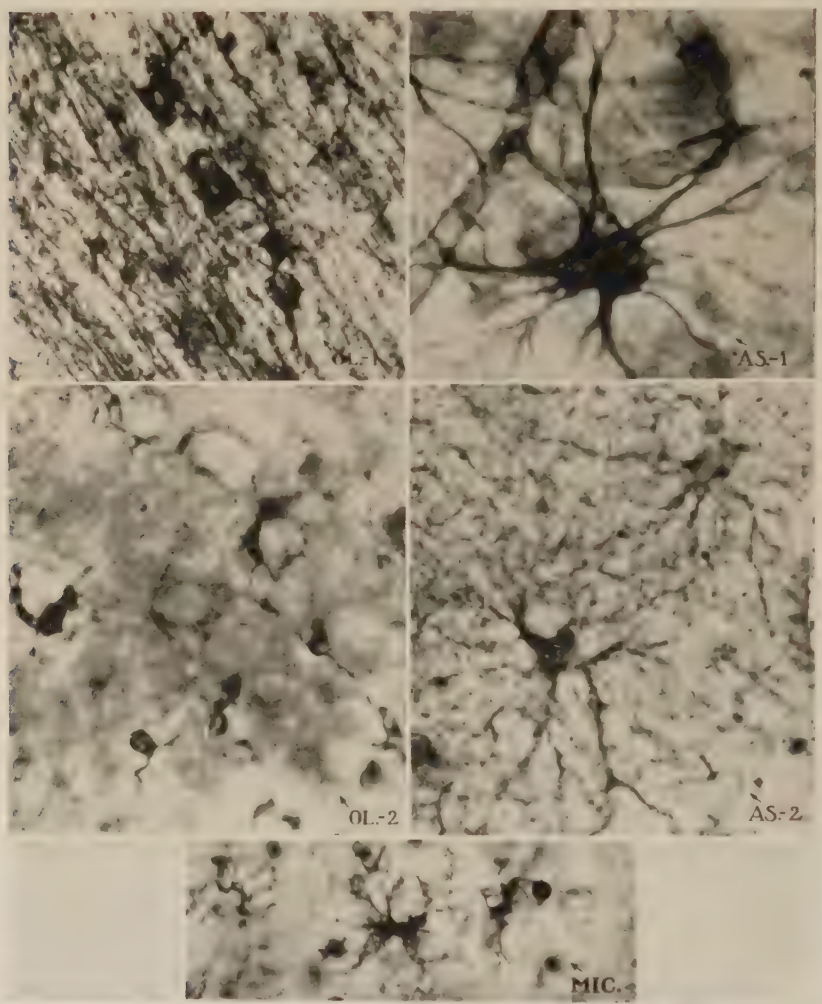


FIG. 487. The interstitial cells: Photomicrographs for comparison with Figure 486. AS-1, fibrous astrocyte with numerous perivascular feet on vessels; AS-2, protoplasmic astrocytes with gliosomes: OL.-1, oligodendroglia in row among medullated fibers (intervascicular glia); OL-2 oligodendroglia about nerve cells which are represented by clear spaces (perineuronal satellites). OL.-1, and AS.-1 from white matter of cerebrum. OL.-2 and AS.-2 from cerebral cortex. MIC., microglia from the gray matter of Ammon's horn with only the nuclei of nerve cells stained. (After Penfield and Cone, 1926*b*.)

at the pole of each cell most distant from the fellow cell, showing that they are the products of cell division. The centrosome is constantly demonstrable

in astrocytes, as in oligodendroglia, and serves to distinguish neuroglia as a group from neurons which lose their centrosomes early (Cajal, 1913*b*) and from microglia in which no centrosome has been demonstrated (Del Rio-Hortega). For a discussion of the centrosome in nerve cells, see page 976.

The *Golgi apparatus* can be demonstrated in the astrocytes of young mammals but not in older subjects. In the young the apparatus is

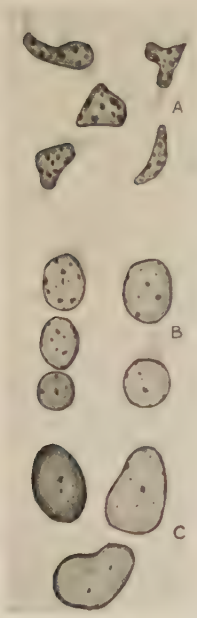


Fig. 488.

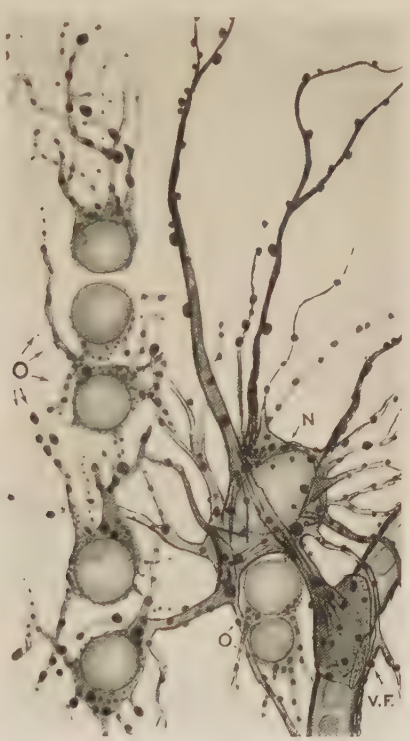


Fig. 489.

Fig. 488.—Nuclei of the interstitial cells from a toluidine blue (Nissl) stain. A, microglia; B, oligodendroglia; C, astrocyte. (After Penfield, 1924.)

Fig. 489.—Gliosomes stained by method of Del Rio-Hortega in cerebral white matter of rabbit. O, oligodendroglia; N, fibrous astrocyte; V.F., perivascular foot. (After Penfield, 1924.)

placed at one pole of the nucleus in the base of the largest expansion. In Cajal's opinion the apparatus is probably perinuclear at a later period, although resistant to staining methods. The Golgi apparatus of young oligodendroglia is similar in form to that of astrocytes.

Pigment granules appear in astrocytes especially in older subjects, or in cases where there has been chronic astrocyte hypertrophy. They are evidently the product of cell degeneration rather than ingestion and may

correspond (Ziveri, 1914) to pigment accumulations in nerve cells (p. 976). Robertson (1897) showed that the pigment is insoluble in ether and does not stain with osmic acid. The pigment is usually collected in groups of globules like grapes or saddle bags on one side of the nucleus. (An account of the pigment in sympathetic nerve cells is given on page 1023.)

Gliosomes are granules described in the protoplasm of neuroglia by Alzheimer, by Ficandt (1910), Nageotte (1920), Cajal (1913*b*), Achúcarro (1913*a*), Del Rio-Hortega (1916*c*) et al. They are present both in astrocytes and oligodendroglia (Fig. 489), in the cell body, expansions and perivascular

FIG. 490.—Cerebral cortex of dog stained by Cajal's gold chloride method. P, pia-mater; M, mixed fibrous and protoplasmic astrocytes. F, fibrous astrocyte; S, marginal glia. Fibers in the perivascular feet on the vein are well seen.

foot (Penfield, 1924). In outline they are clear-cut, and round or a little elongated. In size they vary to a considerable degree and also in stainability (Del Rio-Hortega, 1917). They are more numerous in protoplasmic than in fibrous astrocytes and are particularly numerous in the astrocytes of the human cortex. In fibrous astrocytes they are crowded to the periphery of the expansion by the fiber or fibers.

These granules are stained by certain methods for *mitochondria*. Nageotte believes them to correspond to mitochondria and to resemble, in their development, the secretion granules of gland cells (p. 141). Ficandt and Fisath believed the gliosomes serve to form fibers because the gliosomes are seen to be less numerous in the astrocytes which possess fibers. Del Rio-Hortega (1916*a*), on the other hand, pointed out that in his opinion

the fibrils are formed from the reticulated protoplasm of the cells and not from gliosomes.

The *fibrils* pass through the neuroglia protoplasm (AS.1, Figs. 486 and 487), often being placed at the surface of the cell body, entering it from one expansion and leaving it by another. Weigert (1895), using a specific fibril stain, believed that they became intercellular after formation. He showed very clearly that there is a real chemical differentiation between fiber and protoplasm. More complete methods, however, which demonstrate both fiber and protoplasm, seem to show that under normal conditions the fibers are clothed with a thin covering of protoplasm. The fact that gliosomes accompany these fibers well out toward their end (Fig. 489) is strong evidence in favor of a protoplasmic coating. Fibrils are continued down into the perivascular foot and sometimes stream out over the vessel like a "lock of hair" (Fig. 490F).

It is difficult to exclude emancipation of fiber from cell under all conditions (Da Fano, 1906), but the fibers are certainly formed within the cell cytoplasm and probably continue to bear a coating of protoplasm under normal conditions. There is little evidence of their passing from one cell protoplasm to another.

(*a*) SPECIAL TYPES OF ASTROCYTES

There are certain special types of astrocytes which should be mentioned. Andriezen (1893*b*) described certain "elongated oval fiber cells" which were closely applied to blood vessels. These (a) *perivascular astrocytes* form a meshwork of fibers over the vessel, especially the larger vessels, most of the fibers passing along parallel to the vessel.

Just beneath the cerebral pia is another type of fibrous astrocyte (b), the *marginal glia* cells (Fig. 490S). These cells are applied closely to the under-surface of the pia and send fibrous expansions out parallel to the pia and down into the brain. They may have perivascular feet, but many of these small cells in this reticular layer possess a very robust pial foot, which is often quite short.

In the cerebellum is to be found a special type of (c) *subpial astrocyte*. The nucleus and cell body of this cell are placed in the granular layer near the Purkinje cells, while a long straight expansion passes outward to the pia, thus forming a pial foot. The other expansions are shorter. The pial expansions have been called fibers of *Bergmann*. As will be seen in the description of astrocyte development below, these cells resemble early-formed pial astroblasts.

Cajal (1913*b*) described occasional (d) *satellite astrocytes* (protoplasmic) which are sometimes seen plastered upon nerve cells, their protoplasmic expansions embracing the neuron. Their nuclei are considerably larger than

those of oligodendroglia and their expansions do not resemble those of the latter cells.

Cajal also pointed out that a (c) *mixed type* of *astrocyte* is sometimes seen, particularly in the most superficial layer of the cerebrum in dogs. These mixed cells exhibit some of the attributes of protoplasmic and some of fibrous astrocytes (Fig. 490M). The more superficial expansions contain fibers, while the deeper expansions are shorter, more irregular in shape and are typically protoplasmic in texture.

(*b*) INDEPENDENCE OF ASTROCYTES

Held (1903) described a neuroglial syncytium. It was said to be intercellular and continued into nerve cells as the Golgi apparatus. Held himself admitted that the syncytium was distinct from the glia protoplasm. He

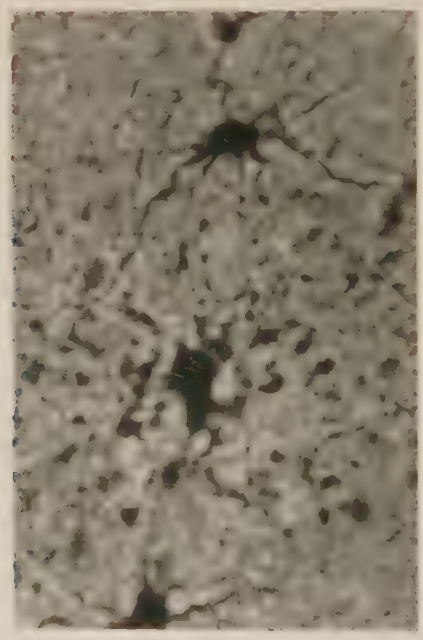


FIG. 491.—Clasmatodendrosis of fibrous astrocytes from human cerebrum. Two astrocytes intact, the other swollen and its expansions fragmented, forming "filling bodies." Cajal's method, photomicrograph. (After Penfield and Cone, 1926a.)

likewise described (1909) a "membrana neurogliae superficialis" and "perivascularis," a limiting membrane made up of neuroglia which was said to separate mesodermal from the ectodermal tissue of the nervous system (see p. 1041).

All of the clearer and more complete methods of staining neuroglia show no reticulum. The illustrations of Held and Hardesty said to demonstrate the reticulum are strikingly indistinct. Achúcarro (1913*a*) produced a clear syncytium and demonstrated that it was an artefact of staining and had no relation to astrocytes. Under pathological conditions the astrocytes are seen to react as units right out to their small expansions (Alzheimer, 1910; Cajal, 1913*a*). This is particularly true in autolytic changes when one cell may undergo an ameboid change while those around are untouched (Fig. 491).

Alzheimer concluded that if a syncytium did exist it must be near the terminations of the expansions. Three of the methods which have been described since his time are even more complete than that of Golgi-Cox, i.e., Cajal's, Achúcarro's and Del Rio-Hortega's. None of these methods shows a syncytium. As will be seen later, the embryonic forms of these cells can also be demonstrated individually. It seems fair to conclude on the morphological, embryological and pathological evidence that, as in the case of the supposed neurofibrillar reticulum, the neuroglial syncytium is an artefact of staining a misleading demonstration of the ground substance.

2. *Oligodendroglia (neuroglia cells without expansions—Nissl, indifferent cells—Bonome, naked nuclei—Schaper, preameboid cells—Rosental, adendritic corpuscles or third element—Cajal, cuboidal cells, perineuronal satellites, indifferent cells):*

Oligodendroglia was first demonstrated by Robertson (1899, 1900*a*) under the name of mesoglia by means of his platinum method. Del Rio-Hortega independently rediscovered the cells by means of a more reliable and complete staining method. He called them oligodendroglia (ὀλίγος—few, little + δένδρον—tree + γλία—glue) because as compared with astrocytes or the astroglia the branches of these cells were small and few and he recognized them as one type of neuroglia. Because of the delicate expansions and the absence of fibers in oligodendroglia, they must fulfill the supportive function of glue to only a very limited extent, if at all. It might be fair, therefore, in the interest of brevity to call these cells oligoglia of Hortega.

There is much in common between oligoglia and astrocytes, as has been shown before. The nuclei are similar, those of the former being rounder and smaller (Fig. 488). They both have centrosomes, have similar Golgi apparatus, similar fuchsinophile granules, similar gliosomes. The outstanding morphological differences are: first, the absence of perivascular feet in oligoglia; second, the absence of fibers; third, the smaller cell body with smaller, more delicate expansions.

There is in oligodendroglia a very marked opposition in the staining reaction of nucleus and cytoplasm. Many methods stain the nucleus but

leave cytoplasm out. Only two methods stain the cytoplasm well and the nucleus is then clear.

The cytoplasm is granular and small in amount. It is often arranged so that the cell appears roughly oblong (OL., Figs. 486 and 487), the expansions arising from corners of the cytoplasm. They are slender, and studded with granules. In the human the cytoplasm is a little more voluminous, the expansions are more ramified and possess larger knees or enlargements at bifurcations than in other mammals.

Although, like astrocytes, oligoglia cells are present throughout the central nervous system, they, too, may be divided into two chief groups, as follows:

(*a*) INTERFASCICULAR GLIA

Interfascicular glia or oligoglia of the white matter. Here the cells are found in rows between the medullated sheaths (OL.-1, Fig. 486). There may be as many as twenty in a row. Scattered in such rows are sometimes intercalated astrocytes and occasionally a microglia cell. The cells in these rows often give the appearance of having undergone direct division.

The longer expansions pass upward and downward upon the medullated tubes and smaller expansions wrap around so that the myelin is enclosed in a loosely woven net of expansions. In the spinal cord and bulb these interfascicular glia cells have larger cell bodies and larger protoplasmic granules than elsewhere.

(*b*) PERINEURONAL SATELLITES

Most of such satellites are oligodendroglia. A smaller proportion is made up of microglia. Microglia satellites are easily distinguished from oligoglia, even when not stained specifically, by difference in the shape of nuclei. The oligoglia satellites often seem to rest in little hollows between neuron expansions. Cajal (1913) observed that he had never seen astrocyte expansions pass between neuron and satellite. The satellite expansions pass around the nerve cell and off toward other structures (OL.-2, Fig. 487).

Thus, as is seen by reference to Figures 486 and 487, the interfascicular glia is seen with the fibrous astrocytes in the white matter and the satellites with the protoplasmic astrocytes in the gray matter. Oligoglia cells, wherever found and whether interfascicular or satellite, have roughly the same morphology, however.

Oligodendroglia also appears throughout both white and gray matter as *perivascular satellites*. The cells are found with the cell body applied to a capillary, while the expansions go off in other directions. Recognition of these satellites is of importance in pathological conditions.

3. *Neuroglia contacts:*

(a) ASTROCYTE AND BLOOD VESSEL

The *perivascular foot* (sucker process, trumpet, podic) was described by Golgi (1885) and denied by Weigert. It contains protoplasm (Fig. 489) difficult to stain by most methods. It wraps around a capillary wall (or small vein or artery). In fibrous cells fibers pass into the footplate and over the vessel. They may also pass on beyond the vessel (AS.-1, Figs. 486 and 487, also Fig. 490).

With regard to the *limiting membrane of neuroglia*, an actual membrane is not seen by selective stains. That there is a potential space (Virchow-Robin) outside the vascular adventitia is well established.

Theoretically the outer boundary of the Virchow-Robin space is formed by the outer layer of invaginated pia mater about the vessel. This layer, according to Held, cements the ectodermal barrier so as to form the limiting membrane. In disease at least this membrane is not impermeable, and cells pass through it freely (Alzheimer, 1910). Also at birth microglia cells (mesodermal) enter the nervous system in large numbers and remain there. Cajal was unable to decide whether the neuroglial foot entered a pre-existing enigmatic substance about the Virchow-Robin space, or whether this appearance was given by coagulated plasma from the space lying beneath. He believed the apparent cement substance was probably plasma.

Ingleby (1925) analyzed the perivascular foot, or podic as she called it, in fibrous astrocytes and found that the "membrana limitans" about capillaries and small veins contained a very fine meshwork of neuroglial fibrils, which meshwork she suggested might well be filled with protoplasm. She called attention to the fact, however, that Weed (1917) caused Prussian blue to pass through this membrane in vivo. Some of the anastomosing fibrils seemed to her to pass through to the endothelium beneath. On the other hand, Achúcarro (1913*b*) was unable to discover fibrils in this wall under normal conditions.

The Virchow-Robin space is demonstrated clearly in pathological conditions when it is seen to be filled with leucocytes or with red blood cells, etc. Under such circumstances the foot processes of the astrocytes are external to the space and doubtless make up, at least in part, this outer wall. It does not necessarily follow from this, however, that there are no mesodermal elements beyond this membrane. The membrane should not be considered as impermeable any more than the wall of a capillary is impermeable.

(b) ASTROCYTE AND PIA

The *pial foot* resembles the perivascular foot. The pia seems to provide a chemotactic influence for astrocyte expansions similar to that of the vessels.

(c) ASTROCYTE AND NEURON

The astrocyte expansions pass about the nerve cells in the vicinity including in this loose lattice the perineuronal satellites.

In general, it is seen that every astrocyte possesses one or more pial or perivascular foot-like processes. Oligodendroglia cells, on the other hand, have no such mesodermal connections. The cytoplasm of the body and expansions of these latter cells is intimately applied to neurons and their medullated expansions, or to blood vessels. They, however, form no specialized terminations.

II. DEVELOPMENT OF NEUROGLIA

1. *Cytogenesis of astrocytes:*

It is generally accepted that neuroglia is ectodermal in origin. The manner of its derivation is of some importance. Early in embryonic life, is formed from the epiblast, the medullary plate. This develops into the medullary canal which, in turn, is represented by the central canal of the spinal cord and the ventricles of the brain.

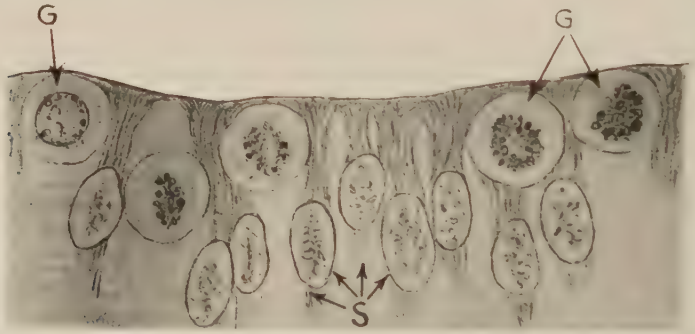


FIG. 492.—The internal limiting membrane of the medullary canal of a human fetus. The large round cells are germinal cells (G), with mitotic figures; the other cells (S), are spongioblasts. (After His, 1889.)

The medullary plate is made up, first, of undifferentiated epithelial cells which give rise to germinal cells (Fig. 492G) and differentiated epithelial cells or spongioblasts of His (His, 1889, 1901). The spongioblasts (Fig. 492S) in their development give origin to neuroglia, while the germinal cells by rapid mitotic division produce neuroblasts which, emigrating from the epithelial layer, form nerve cells. Schaper (1897) pointed out that the germinal cells are likewise capable of producing spongioblasts, to which His (1901) agreed. Schaper felt that these indifferent cells remained undifferentiated for a long period and preserved the potential ability to develop either into neuroblasts or spongioblasts.

The spongioblast at an early period in embryonic life sends out an expansion which terminates at the external limiting membrane or pia mater, while at the other pole the cell is applied to the internal limiting membrane or the membrane of medullary canal (Fig. 493). This inner portion develops cilia which project into the canal. The end of the outer expansion is enlarged into a footplate beneath the pia. These ependymal cells (ependymal glia, spongioblasts) form the only supportive structure at one stage of embryonic development.

The spongioblast then migrates outward and in time loses its connection with the medullary canal, thus being converted into what Cajal (1909)

FIG. 493.—Spinal cord of ten day chick; a, ependymal cell or spongioblast; b, spongioblast whose cell has emigrated; c, astroblast (displaced epithelial cell, Cajal). Disappearance of the pial connection would finish the metamorphosis to astrocyte. (After Lenhossék 1895.)

called a displaced epithelial cell and Lenhossék (1895) an astroblast (Fig. 493). Further evolution may cause disappearance of the pial expansion and development into an adult astrocyte. In the adult human central nervous system examples of all these primitive forms are preserved, i.e., the ependymal cell (spongioblast with attachment to pia and central canal) in the median raphé of the hindbrain and in the ventral sulcus of the spinal cord; the subpial or marginal glia has retained its pial foot as has also the cerebellar astrocyte with its very long pial process (fiber of Bergmann).

It has been strongly urged that although the germinal cells are clearly individual at first they come to form a reticulum from which neuroglia is developed (Hardesty, 1904; Streeter, 1912). Cajal (1909) maintains that

these cells are always independent, as is shown very clearly by Golgi's chrome silver method. The question of reticulum is taken up elsewhere in this section (p. 1040).

In the adult the spongioblasts develop into simple ependymal cells and the primitive epithelium is represented by the ependyma which lines the central canal of the spinal cord and ventricles.

In human embryos at about the third month of intrauterine life neuroglia astrocytes begin to appear. From the fourth month on, fibers of Ranvier-Weigert begin to be found in these cells. The fibers are formed first in a

FIG. 494. Cerebral cortex of kitten of fifteen days; a, subpial astrocyte (marginal glia); D, vessel coming in from pia about which are seen astroblasts. (After Cajal, 1913.)

large expansion and reach the cell body secondarily (Da Fano, 1906; Del Rio-Hortega, 1917). But at birth, both in man and in the small mammals (as dog, cat, rabbit) the astrocytes are immature and contain few fibers. They typically possess a long, robust vascular process, while the other processes are short, feathery and protoplasmic (Fig. 494).

New-formed spongioblasts are still migrating at this stage from the ependymal epithelium (Fig. 495), only now the peripheral expansion becomes attached to a vessel (D, Fig. 495) rather than to the superficial pia mater.

The invasion of the nervous system by the vascular tree with its investment of pia has provided a new attraction surface for the peripheral pole of spongioblast. Thus the young astrocytes (Fig. 495) which are formed when

the central attachment is dissolved, correspond exactly with the astroblasts of Lenhossék in Figure 493. It seems well, therefore, to call them astroblasts, as Bailey and Cushing (1926) have done.

As the embryo grows, the number of astrocytes increases enormously, and it seems apparent that the primitive method of cell evolution can hardly be responsible for such an increase. Because of this, Lenhossék (1895) urged that the germinal cells give rise to astrocytes both in the vicinity of the central canal and after wandering to a distance from it, although he admitted that at a distance from the ependyma he had not identified the mitotic forms by which the germinal cells are said to multiply.

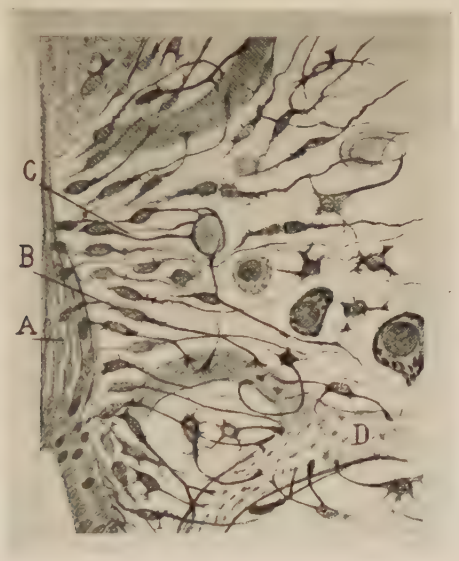


FIG. 495.—Transverse section of the spinal cord of a kitten of eight days. A, epithelial cells in the posterior raphe; B and C, dislocated epithelial corpuscles whose peripheral expansion is attached to a capillary; D, capillary to which are attached young astrocytes. (After Cajal, 1913.)

Kölliker (1890) believed that the supplementary source of astrocytes was undifferentiated spongioblasts which maintain their early rounded form and increase by direct division till relatively late periods. He suggested the name "indifferent cells of the medullary anlage." Cajal (1909) inclines to the opinion that astrocytes are developed only from spongioblasts and not from wandering germinal cells.

Wandering germinal cells (indifferent cells of Schaper, medulloblasts of Bailey and Cushing, 1926) were said by Schaper to wander out into the mantle layer and there divide by mitosis, giving rise both to neurons and astrocytes. Cajal, on the contrary, was never able to discover in the chick embryo these indifferent cells described by Schaper. Also, contrary to what might be expected of Schaper's cells, the cells which are developing into astrocytes at about the time of birth are themselves rapidly proliferating by *direct division* (Cajal, 1913*b*). In that respect and in form, as will be shown below, these pre-astrocyte cells resemble spongioblasts rather than germinal cells.

2. Cytogenesis of oligoglia:

These cells are not present in early embryonic life but they appear in later embryonic life and are present in large numbers in an embryonal form about the medullary canal during a period which antedates the first appearance in the embryo of microglia (Del Rio-Hortega, 1921*b*). They increase rapidly at about the time of birth and remain practically constant in number through adult life. Thus at birth they are present throughout, particularly in the white matter, and the cell cytoplasm is more voluminous than that of adult oligoglia. The protoplasmic granules or gliosomes are very large at this period, a fact which Del Rio-Hortega associates with the very active formation of myelin which is taking place at birth.

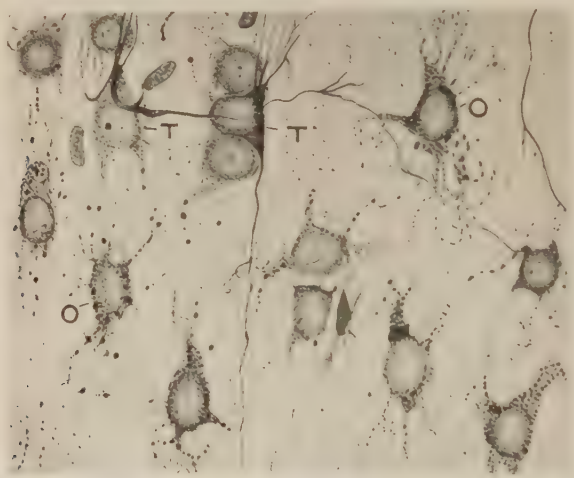


FIG. 496.—Cerebellar white matter of eight day kitten. O, young oligodendroglia; T, immature astrocyte with some expansions, still resembling the young oligodendroglia. Silver carbonate method. (After Penfield, 1924.)

Hardesty (1905) has described small cells which appear on medullating fibers in the central nervous system. His illustrations leave little doubt that he was describing oligodendroglia cells. The cells sometimes presented signet ring forms in which the cytoplasm is found wrapped around a nerve fiber. He observed that such cells were never present before the onset of myelination and fibrous neuroglia appeared only after this process was well under way. He was unable to stain these "signet ring" forms at a later period of development.

In young mammals, in addition to the formation of astrocytes by attachment of spongioblasts to vessels, there are many migratory spongioblasts which do not form such attachments (Cajal, 1913*b*). These cells migrate into the white and gray matter and there develop into oligoden-

droglia or they may even then form astrocytes, as shown by the intercalated astrocytes that frequently appear interspersed in the long rows of oligoglia (Del Rio-Hortega, 1921*b*). These intercalated cells as well as the "third element" cells are not seen until late embryonic life (Cajal, 1913*b*).

When myelination is at its height fibers can be seen appearing (Fig. 496) in the expansions of an occasional young oligodendroglia cell or oligodendroblast (Penfield, 1924). These cells develop into astrocytes and become the intercalated cells among the oligoglia.

In the spinal cord of a young kitten the white matter is packed densely with cells (Fig. 497) whose nuclei are small and round and resemble those of the spongioblasts which quit the medullary epithelium at about that

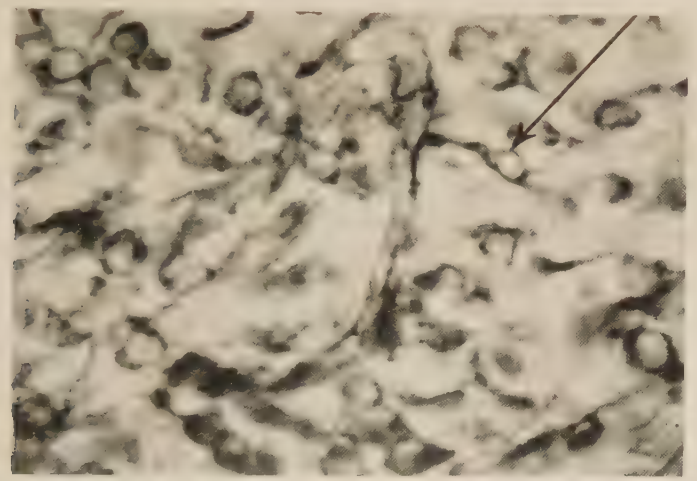


FIG. 497.—Photomicrograph, substantia blanca, spinal cord of eight day kitten. Young oligodendroglia cells (oligodendroblasts) present in large numbers. A, transition form from oligodendroblast to astrocyte. This cell and a few others are drawn in Figure 15. Silver carbonate stain, magnification × 680.

period (Fig. 495). The cytoplasm is scanty and is collected into one or two nipple-like projections from which may arise fine short irregular expansions containing granules (*o*, Fig. 498). These cells have no vascular attachments and they are multiplying by direct division. All stages of transition can be seen from them to astroblasts, and at the earliest sign of differentiation toward the astrocyte a vascular foot appears (A, Figs. 497 and 498) which is the indispensable prerequisite (Cajal, 1913*b*) to the formation of neuroglia fibers.

Thus, it is apparent that emigrant spongioblasts develop either into astrocytes or into oligoglia. The nuclear size and chromatin arrangement of the spongioblast, the oligoblast and the astroblast are very much the same.

It is of interest that a *phylogenetic study* of neuroglia discloses the same forms as are seen in its ontogenetic development. In the earliest organisms the supportive structure is formed by the ependymal cells which send expansions to the outer surface of medullary substance, as in amphioxus (Lenhossék, 1895). Farther up the phylogenetic scale there appear also the astroblasts with pial attachment but no connection with the medullary canal (e.g., cyclostomes). These forms possess no intramedullary blood vessels and no medullated fibers. Autonomous glia (astrocytes with no ependymal or pial attachment) appear first in any considerable number in the plagiostomes (Kappers, 1920), and the nervous system of these forms is provided with internal vessels and medullated sheaths. Achúcarro (1915) found that among the forms which he examined a well-developed vascular

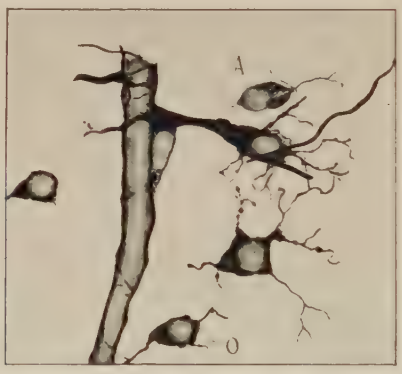


FIG. 498. Drawing to show more completely some of the cells photographed in Figure 497. A, transitional cell, neurone, showing perivascular foot and some fibers. The other cells illustrate stages from spongioblast to oligodendroblast.

foot process, resembling that seen in birds and mammals, first made its appearance in the lizard. Also in this form he found the first evidence of the "third element of Cajal" by which term he probably referred to oligodendroglia. He pointed out that when the astrocytes become autonomous the neurons cease to be juxta-ependymal.

Thus it appears likely that both in phylogenetic and ontogenetic development the autonomous astrocyte first appears, about the time of invasion of the nervous system by blood vessels, when the perivascular foot is substituted for the pial foot. Likewise it is of interest to suggest that the oligodendroglia cell appears at the time of first myelination, although further studies are necessary to prove this relationship completely. In the increasingly complex nervous system the varying forms of glia are evidently produced by varying adaptations of the same type of cell, the spongioblast.

Reference to the schema (Fig. 499) will serve as a recapitulation. In *panel I* is seen the development of the earliest supportive structure, the ependymal cell or spongioblast (s) with attachment to internal and external limiting membrane. In *panel II* is illustrated the outward growth of the primitive spongioblast (s) and its conversion into the astroblast of Lenhossék (ab) by disappearance of its central connection; also the development of this astroblast into an autonomous astrocyte (ac) by the conversion of the pial expansion into a vascular attachment. *Panel III* illustrates the development of spongioblast into astroblast (ab) and astrocyte (ac) by direct attachment to a blood vessel and without the interpolation of an attachment to the now distant pia. *Panel IV*, in the period of myelination, illustrates the germinal cell giving rise to emigrant spongioblasts which are converted into astrocytes by the formation of a vascular attachment, or into young oligodendroglia (oligodendroblasts, ob) and oligodendroglia (odg).

The whole process of formation of oligodendroglia demands further study and one must maintain an open mind for the present concerning the rôle of the indifferent cell of Schaper, but we have not seen such cells leave the epithelium at the stage of myelination.

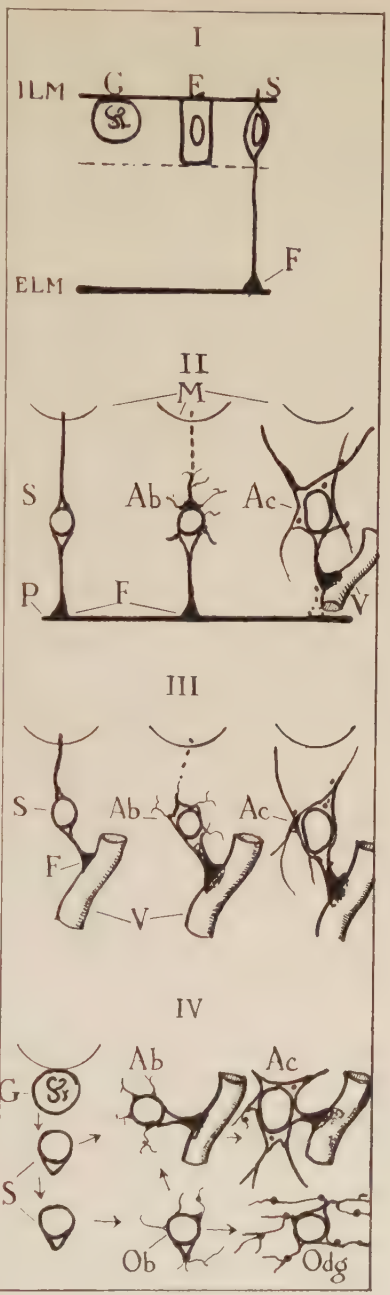


FIG. 499.—Schema of development of neuroglia. I. G, germinal cell; E, epithelial cell or primitive spongioblast; S, supportive spongioblast or ependymal cell; ILM, internal limiting membrane; ELM, external limiting membrane. II. S, supportive spongioblast; Ab, astroblast; Ac, astrocyte; V, vessel; P, piamater; F, pial foot. III. S, supportive spongioblast; F, perivascular foot; Ab, astroblast; Ac, astrocyte; V, vessel. IV. S, emigrant spongioblast; Ab and Ac and G, as above; Ob, oligodendroblast; Odg, oligodendroglia.

The spongioblast, on the other hand, in little differentiated form, seems to emigrate in response to some type of activity within the neuron itself. The cells place themselves about the nerve cell body, particularly at the base of the axon, and along the nerve fibers. At that time myelination begins and the cells take on the characteristics of oligoglia. The spongioblast, even after its evolution into the oligodendroblast, may still develop into an astrocyte forming a vascular attachment.

III. OVERGROWTH AND DEGENERATION OF NEUROGLIA

These changes are of considerable pathological interest but they will only be touched upon very briefly here. Neuroglia response to unfavorable conditions is, in general, degenerative or hyperplastic.

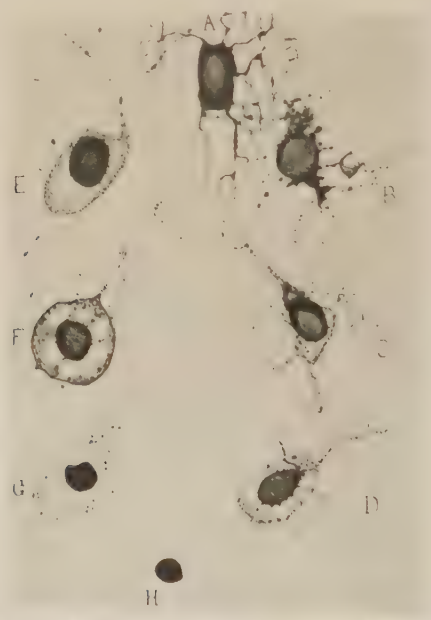


FIG. 500. Acute swelling of oligodendroglia illustrating various stages in the transition from the normal to cell destruction. A is to be regarded as the normal form and B-H as the abnormal, or in other words, as stages in the process of acute swelling.

1. *Degenerative changes:*

(*a*) ACUTE

Alzheimer (1910) described an acute alteration of neuroglia which he called the *ameboid* change characterized by fragmentation of cell expansions and the appearance in those fragments (Füllkörperchen) and in the cell

protoplasm of methyl blue granules. Cajal (1913*b*) described a similar change (Fig. 491) as a post-mortem autolytic alteration. He called the process *clasmatodendrosis*. It has since been pointed out by numerous workers that the two processes are really the same.

The ameboid change in astrocytes may be caused by acute affections of the central nervous system preceding death, or the change may be produced locally during life by a local ischemia. It also occurs as an autolytic change after death and it sometimes takes place within a few hours. At other

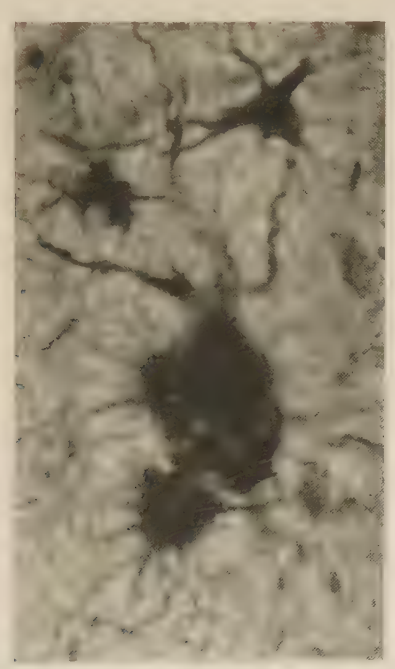


FIG. 501.—Monster cell in cerebral cortex of a boy of eight years. The alteration in the astrocytes is due to chronic progressive vascular occlusion.

times clasmatodendrosis fails to appear even after twenty-four to forty-eight hours. It typically occurs unevenly throughout the central nervous system, one cell being spared and the next destroyed (Fig. 491). The best evidence indicates that these ameboid cells are *not* transformed into mobile macrophages.

The same causes produce a change, also, in oligodendroglia, "*acute swelling of oligodendroglia*" (Penfield and Cone, 1926*a*). This change is characterized by hydropic swelling of the cell with fragmentation of its extremities (Fig. 500). The process may go on to cell destruction or it may stop, as it seems to be reversible. The oligoglia alteration takes place

much earlier (pre-ameboid change of Rosental) than that of astrocytes. It is present at death in the nervous system of practically all human cases dying in coma. When present ordinary stains may show a clear space about the nucleus which has erroneously been taken to be characteristic of normal human oligoglia.

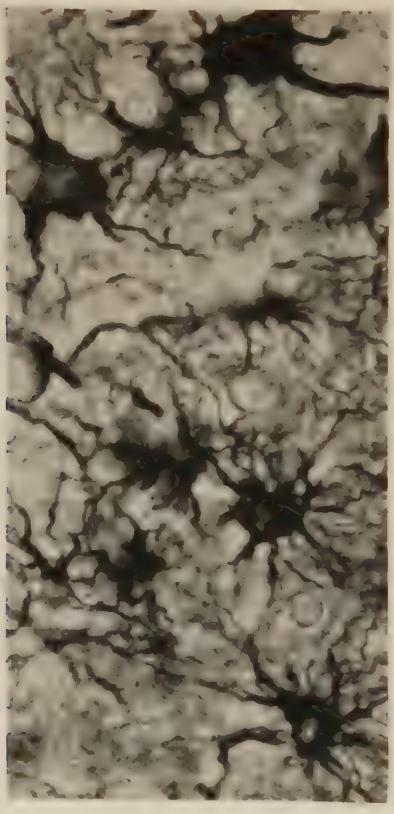


FIG. 502.—Gliosis in cerebral white matter. Same case as Figure 501.

(*b*) CHRONIC

Chronic unfavorable conditions, if they do not cause clasmatodendrosis, may result in a chronic degenerative form of astrocyte, the *monster cell*. The body and expansions of these cells become very large (Fig. 501) and the nuclei often become multiple and take on bizarre shapes. The cells sometimes appear to divide so that the change may be both degenerative and hyperplastic, but the end result is the gradual loss of cell expansions associated with the phenomenon of dendrophagocytosis.

2. *Hyperplastic change:*

(*a*) GLIOSIS OF ASTROCYTES

Oligodendroglia cells play no part in this reaction so far as has yet been determined. A very common response to chronic pathological lesions in the central nervous system is gliosis. It is so frequently seen that Weigert considered it the invariable result of pathological conditions. By gliosis is meant the hyperplasia and hypertrophy of astrocytes associated with fibrous change in these cells. Swelling and direct division of cells (Del Rio-Hortega and Penfield, 1926) is followed by hypertrophy and the formation of glia fibrils regardless of whether the cell was originally protoplasmic or fibrous (Fig. 502). This takes place, to a certain extent, as a senile change as well as in pathological conditions of the brain.

(*b*) SATELLITOSIS OF OLIGODENDROGLIA

Inspection of sections of normal tissue shows lines and pairs of oligodendroglia which give the appearance of having undergone direct division. Factors which may be responsible for hyperplasia of these cells have not yet been clearly worked out. We have seen pathological cases, however, in which specific stains showed the perivascular and perineuronal oligoglia satellites to be definitely increased (satellitosis: see Marinesco, 1909). Thus in certain cases, at least, of so-called neuronophagia multiplication has taken place in the oligoglia. From this satellitosis a perivascular and even a perineuronal leucocytic infiltration must be carefully distinguished.

IV. MICROGLIA, OR MESOGLIA OF HORTEGA (HORTEGA CELLS, THIRD ELEMENT OF THE CENTRAL NERVOUS SYSTEM, ADENDRITIC CELLS, ETC.)

In the central nervous system of normal subjects microglia cells were never stained in their entirety previous to the introduction of Del Rio-Hortega's method (1920). The small elongated nuclei had of course long been observed and Nissl's toluidine blue method demonstrated a small amount of protoplasm at the base of what seemed to be rudimentary expansions. Nissl (1904) also observed that in general paralysis of the insane his method brought out rod-like cells with elongated nuclei ("Stäbchenzellen") and the presence, under pathological conditions, of large, compound granular corpuscles has long been observed.

Both the rod cells and compound granular corpuscles are derived from microglia, as will be described below. Thus Del Rio-Hortega's description of the cell from which these pathological forms arise is a long step toward the simplification of neuropathological reactions.

Microglia was included with oligodendroglia in the group of so-called cells without expansions which Cajal (1913*b*) had named the third element of the central nervous system. It is to microglia cells alone that Del Rio-Hortega would apply the term third element, and it will be seen that because of their different origin and function they do form a *third element* in the central nervous system.

In some ways the third element of Hortega or mesoglia of Hortega (Cajal, 1920*b*) is a more descriptive term than microglia because, as Metz and Spatz (1924) pointed out, compound granular corpuscles are large and thus when the cells assume this form microglia is a misnomer. Mesoglia of Hortega, however, must not be confused with the mesoglia of Robertson, for the latter cells were the same as oligodendroglia (Penfield, 1924).

1. *Morphology:*

The mesoglia cells of Hortega are scattered through the central nervous system of man and of all animals so far investigated. The cells are more plentiful in gray than white matter and the form, which is practically the same in various types of mammals, depends largely on environment. It is perhaps because of the fact that the nervous system is made up structurally of a large number of units shaped like tubes, wires and balls, that the granular structureless cytoplasm of mesoglia is distributed in slender complicated irregular expansions

The *nucleus* is usually elongated or roughly triangular. It resembles the nuclei of connective tissue and is easily distinguished from the other interstitial cells by its shape and small size (Fig. 488). The nuclear chromatin, also, as illustrated in Figure 488, is heavier.

The finely granular *cytoplasm* contains no gliosomes nor have mitochondria of any form nor centrosomes yet been demonstrated in the cell. Mesoglia does not form fibers and never has a perivascular foot. The expansions are tortuous and bear small terminal spines set upon them at right angles (Mic., Figs. 486 and 487).

2. *Types:*

In the gray matter mesoglia cells make up the less numerous portion of the *perineuronal satellites* (Mic., Fig. 487). The cell body is often plastered upon the nerve cell, while the expansions go off to a distance passing loosely about other neurons.

Also, like oligodendroglia and the astrocytes of Andriezen, mesoglia cells are frequently found on vessels (*perivascular satellites*). They are outnumbered on the vessels also by oligoglia.

In the medullated tracts Hortega's mesoglia cells are less numerous than in gray matter. They are tucked in between the medullated tubes and even occasionally insinuated into rows of oligodendroglia.

V. ABNORMAL FORMS OF MESOGLIA

1. *Rod cells:*

Nissl (1904) was the first to give these cells a clear description while Del Rio-Hortega (1920) demonstrated the transitions between microglia and rod cells. The cytoplasm of normal mesoglia becomes somewhat swollen and elongated, lateral expansions being replaced by short stubs. These forms are particularly numerous in general paralysis. When there is a frankly destructive lesion to the brain, the change goes farther, resulting in compound granular corpuscles.

2. *Compound granular corpuscles:*

Experimental brain wounds provide the best opportunity to study transitions between normal mesoglia and macrophages of the brain (Del Rio-Hortega, 1921*a*; Penfield, 1925*b*). The mesoglia in the vicinity of the wound

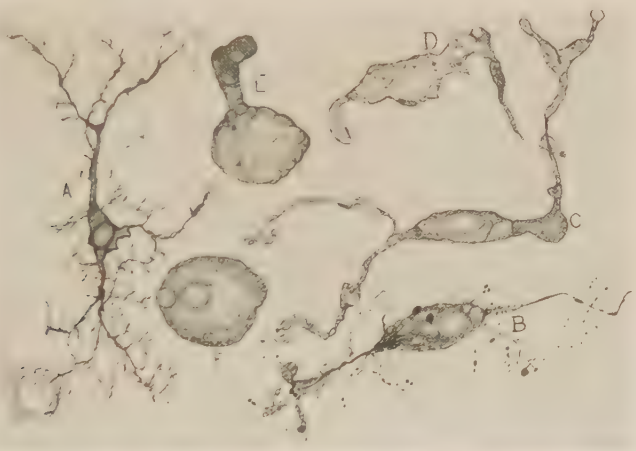


FIG. 503.—Stages in the transition of normal microglia (A) to compound granular corpuscle (F) from the human brain in the vicinity of a glioma. (After Penfield, 1925*a*.)

swell, lose their expansions and, passing through a stage which resembles the rod cell, become compound granular corpuscles as early as twenty-four to forty-eight hours. During this metamorphosis the cells migrate toward the site of injury. The stages in the ameboid transformation of mesoglia may be seen also in the vicinity of other destructive lesions of the brain such as an area of softening, and expanding tumor, etc. (Fig. 503).

Infections of the nervous system which call out leucocytes from the blood stream do not cause the formation of compound granular corpuscles unless the infection goes on to destruction, although there may result lesser changes in mesoglia (Cone, 1925).

3. *Origin of the abnormal forms:*

Alzheimer (1910) considered "Gitterzellen" (compound granular corpuscles) to have a double origin: first, from ameboid glia and, second, from a mesodermal source. Nissl (1904), as well as Alzheimer, considered Stäbchenzellen to be of mesodermal origin and Nissl believed in the same source for Gitterzellen. Selective staining methods developed more recently show: first, that there is no transition from ameboid glia to the mobile macrophage (Del Rio-Hortega, 1918; Penfield and Cone, 1926; see also excellent discussion after different staining methods, Wohlwill, 1914); second, the ameboid change ends in death to the astrocyte (Cajal, 1913*b*); and third, the compound granular corpuscle is developed directly from Hortega's mesoglia and apparently from certain other mesodermal sources as well (Del Rio-Hortega, 1921*a*). In addition to those cells derived directly from ramified mesoglia they may arise from other mesodermal sources, such as large mononuclears and perhaps the adventitia of vessels. Compound granular corpuscles are also produced by mitotic division (Penfield, 1925*a*).

VI. THE MESODERMAL ORIGIN OF MICROGLIA

Virchow (1867) discovered ameboid cells, which he called "Körnchenzellen" in the central nervous system of newborn infants. As the cells contained fat he considered that they indicated a fatty degeneration and he called the condition congenital encephalitis. However, he admitted that the condition was not invariably fatal. He found that these cells were confined largely to the white matter and that when they were studied with dark ground illumination in teased specimens, the cells in question appeared to be actively moving about. Boll (1874) also described movable cells in the embryo which he thought to be immigrating mesodermal neuroglia cells. Thus immigration of mesodermal cells into the embryonic nervous system is not a new conception but the historical aspects of this subject must be omitted here.

The following discussion is based largely on the work of Del Rio-Hortega, and others who have used his method which is specific for mesoglia and which demonstrates both the developmental and pathological forms of these cells.

1. *First appearance:*

Del Rio-Hortega (1921*a*) demonstrated the ameboid cells, described by Virchow to be young microglia. He found that no mesoglia in any form was present in embryos until after the development of blood vessels within the central nervous system. In the rabbit, the mouse and the rat he found that the above ameboid cells appear just before term and that their number

increases slowly up to the fourth day after birth. From that time on the ameboid forms disappear rather rapidly.

2. *"Fountains":*

Hortega found certain areas where these cells appeared first and in large numbers. There are three of these zones in the brain which he called fountains of microglia. One is at the point where the invagination of the pia forms the tela choroidea of the third ventricle. Here the pia mater is applied

FIG. 504.—Transverse section of brain of rabbit of four days. J, tela chorioidea; G, fimbria; A, corpus callosum. Newly formed microglia above and below tela with somewhat older forms at a distance. (After Del Rio-Hortega, 1921*a*.)

to the corpus callosum and fornix above and the thalamus and third ventricle below. It is found that subjacent to this layer of pia at the time of birth there is a zone of ameboid cells which stain selectively by the microglia method (Fig. 504). In the adjacent fimbria and corpus callosum can be seen many similar cells which possess pseudopodia and give every morphological evidence of migration.

The pia mater is not stained simultaneously. It is not possible, therefore, to describe transitions in the cells of this membrane. However, the most superficial cells are flattened out and are more like the cells of the pia in form than those which are further removed. If these cells, with the pseudopodia, are not being formed at this surface, they must be approaching it from some other source, and it seems difficult to conceive of their having

FIG. 505. Photomicrograph of "fountain of microglia" beneath tela chorioidea superior of rabbit of one day. The unstained zone is pia. Just beneath it are young mesoglia. (Zeiss, a 2mm. apochromat, magnification × 546.)

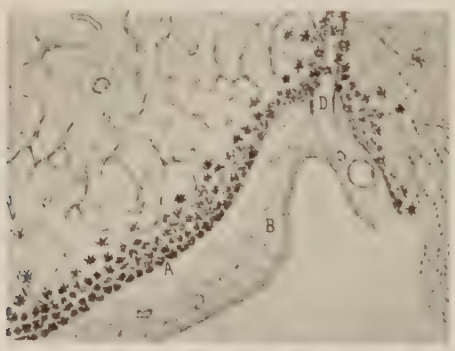


FIG. 506. Peduncular region, brain of rabbit of four days. A, new formed globular microglia, B, pia mater; D, vessel. (After Del Rio-Hortega, 1921a.)

been attracted from a diffuse origin to this area of the brain in which at later periods these cells are not to be found in any form.

The evidence seems conclusive, therefore, that ameboid cells are being formed here beneath the pia. They can be photographed, though incompletely (Fig. 505), and are seen to be migrating away from the under-surface of the pia. The fact that elsewhere, as beneath the ependyma of the free ventricle, no such concentration of these cells is seen is evidence also, though negative, in favor of the pia as the source of these cells.

Another fountain is situated in the vicinity of the tela of the fourth ventricle and yet a third is found in a zone just beneath the pia mater of the cerebral peduncles (Fig. 506). In the spinal cord of newborn mammals these cells are found in a similar relation to the pia, particularly along the ventral and dorsal sulci.

Clumps of ameboid cells are also found at this time about vessels. They may perhaps be following along the vessel in the perivascular space, but the fact that they are more often seen about the larger vessels is taken by Del Rio-Hortega for evidence in favor of their formation from the pial lining which is better developed about such vessels.

3. *Immigration:*

The young mesoglia cells enter the myelinating tracts and spread rapidly along the clefts in the white matter by means of ameboid movements (Fig. 507). Thus in the early days after birth all of these cells are in the white matter and none in the gray. They reach the cerebral cortex (cat, dog, rabbit) on the fourth to the sixth day after birth and increase in amount in this situation up to the fifteenth day. They also appear early in the basal ganglia and olfactory lobe. In the spinal cord mesoglia is all peripheral at first and reaches the central portion later.

Upon reaching the gray cortex the cytoplasm of these cells elongates into multiple complex expansions so that microglia of adult, normal form is to be seen in certain areas, while migration is still continuing along the main fiber tracts. The stages in the development of a microglia cell as described by Del Rio-Hortega are as follows: (1) tuberous (containing vacuoles), (2) ameboid or pseudopodic (containing protoplasmic inclusions), (3) ramified (as seen in normal adult).

Finally the *location of the fountains* mentioned above is of considerable interest. They are situated in the only three areas of the brain where pia mater comes into close contact with white matter. The tracts of the white matter are myelinating at the period of mesoglia invasion and it may be suggested that some product of this chemical activity has a chemotactic influence upon the lowermost layer of pial cells. The influence may not be exerted so strongly through the mantle of gray matter which covers the brain everywhere else. This might explain why mesoglia is not formed

elsewhere beneath the pia and why clumps of new-formed cells seem to arise from the investment of some vessels and not from others.

These migrating cells may contain fat and protoplasmic inclusions, and it is possible that they play some rôle secondary to the process of myelination, probably in the nature of that of a macrophage. As the cells assume

FIG. 507.—Immigration of newly formed mesoglia in the corpus callosum of a rabbit of one day. Photomicrograph, magnification × 546.

the ramified shape, they seem to enter upon a resting phase and remain in this phase until perchance they are acted upon by some product of brain degeneration when they rapidly revert to a form similar to that in which they first appear in the nervous system.

4. *Morphological evidence:*

The mesoglia nucleus (Fig. 488) differs in shape and amount of chromatin from the nuclei of neuroglia. Its elongated shape resembles the nuclei of

connective tissue. No centrosome or Golgi apparatus has yet been demonstrated in any of the forms of mesoglia, in contrast to neuroglia and neurons. Mesoglia contains no gliosomes and forms no fibers or vascular attachments. Thus, morphologically there is no evidence in favor of an origin for microglia in common with the other cells of the central nervous system.

5. Biological evidence:

Acute agonal and post-mortem conditions which cause dissolution of both forms of neuroglia do not affect microglia. On the other hand, focal brain destruction produces rapid ameboid change in microglia. It becomes phagocytic and multiplies rapidly by *mitosis*. If not destroyed by the lesion, astrocytes multiply rapidly by *direct division* (Hortega and Penfield, 1926) and lay down fibers in their protoplasm.

Mesoglia bears much resemblance in its activity to macrophages elsewhere in the body (Del Rio-Hortega and Asua, 1921). The mesoglia staining technique demonstrates macrophages everywhere more or less selectively and picks out the cells of Kupfer in the liver. Metchnikoff included neuroglia and neurons among the phagocytic fixed cells and Cerletti (see Marinesco, 1909) showed that both these types of cell ingest India ink particles.

Mesoglia, on the other hand, may be included in the large group of cells called the reticulo-endothelial system (Aschoff, 1924) (p. 428). In general paralysis of the insane all of the phagocyted iron pigment is found in mesoglia and the adventitial cells of the vessels (Metz and Spatz, 1924). Ingestion of iron is a characteristic of cells of the reticulo-endothelial system. Renaut believed the mobile macrophages of the body were capable of transformation into connective tissue with subsequent return to their previous form in the presence of a pathological condition. Whether or not that be so, mesoglia passes from an ameboid embryonic form to the ramified form and under the certain pathological conditions returns to an ameboid state again.

All authors who have worked on the subject with specific stains (Del Rio-Hortega, Cajal, Metz and Spatz, Marinesco and Lupa, Penfield) have agreed to the transformation of these cells into compound granular corpuscles. All of them have expressed the opinion that the cells are of mesodermal origin, except Metz and Spatz who felt that the ramified mesoglia arose from astrocytes, their study being on pathological material alone, however.

Finally, the developmental, morphological and biological evidence is in favor of the mesodermal nature of microglia which may therefore be called *mesoglia* of Hortega.

VII. FUNCTION OF THE INTERSTITIAL CELLS

1. *Astrocytes:*

Neuroglia astrocytes have long been considered the supporting structure of the nervous system, and it seems evident that the fibrous astrocytes with their robust fibrils and their pial and vascular attachment must perform a function subserved in other organs by connective tissue. This becomes more evident when it is realized that the blood vessels and pia form the only continuous skeleton-like structure in the nervous system.

It has been suggested also that astrocytes serve the neurons in a nutritive and insulating capacity, and the possibility has even been put forward that, by contraction of their expansions they may make and break nerve contacts at synapses (Cajal). At all events, in pathological conditions these cells do play an important rôle in cicatrix formation.

Of recent years the view that the astrocytes form a gland of *internal secretion* (see gliosomes, p. 1038) has been proposed for the following reasons: (a) they are applied by their expansions to vessels; (b) they contain gliosomes which resemble secretion granules, and the amount of protoplasm is large, particularly in the human gray matter; (c) the form and stainability of gliosomes varies, suggesting variations in functional activity; (d) the so-called glandular cells of the epiphysis are neuroglial in nature; (e) changes in astrocytes suggesting variations in physiological activity have been reported to follow the administration of thyroid extract, pilocarpine, etc. (Del Rio-Hortega, 1917). Some doubt is cast upon the glandular significance of gliosomes by the fact that they are found also in oligodendroglia cells which have no perivascular foot processes and thus do not possess the supposed apparatus for the discharge of a hormone.

2. *Oligodendroglia:*

The perineuronal satellites were suggested by Cajal to be living in perfect symbiosis with nerve cells, and he compared them with the subcapsular cells of the spinal ganglia. Del Rio-Hortega (1922) pointed out that the relationship between oligodendroglia and the myelin sheaths within the central nervous system bore a striking resemblance to the relationship maintained by the sheath of Schwann cells with peripheral nerve fibers. He pointed out also that the medullated tubes of the cord occasionally show markings similar to the incisurae in the peripheral myelin sheath.

The evidence in favor of the formation and maintenance of myelin by oligodendroglia is briefly as follows: (a) oligoglia first appear in the fiber tracts in large numbers at the time of myelination; (b) they are never seen before myelination begins; (c) at this early period the protoplasmic granules are unusually large and numerous and they contain gliosomes at all times; (d) the position of the cells is in rows between the medullary tubes

resembling the arrangement of sheath of Schwann cells; (e) the perineuronal satellites resemble the subcapsular cells of the spinal ganglia in their relation to the neuron; (f) the expansions are always protoplasmic and wrap around the nerve tubes; (g) oligodendroglia cells follow nerve tubes right up to the cell body and are especially liable to be placed on the sides of the axon hillock.

Certainly oligoglia must play a rôle directly secondary to that of the neuron and it seems likely that it is these cells which enable the neuron to maintain perfect functional activity over so great a length of cell continuity, a distance of several feet in the case of the Betz cells.

3. *Mesoglia:*

The function of these cells has already been pointed out. In the ameboid form they act as phagocytes, doubtless submitting ingested substances to some digestion. They seem at times to bear their contents to the vessels in the neighborhood where they are found closely applied to the vessel wall. It may be, also, that the same cell returns from the vessel empty.

Ameboid mesoglia have a specific function with regard to the large fibrous astrocytes which are undergoing chronic degeneration. Mesoglia cells apply themselves to the expansions and to broken fibers, wrapping themselves about such fibrillary bits but avoiding the cell body (*dendrophagocytosis*) (Penfield, 1925). The astrocyte body seems to be immune, as mesoglia is found in contact with the degenerating expansions only.

Whether or not the ramified form of mesoglia in the normal brain serves any function is purely a matter for conjecture.

VIII. BIBLIOGRAPHY

Achúcarro, N. 1911. Neuroglia y elementos intersticiales patologicos del cerebro, impregnados por los metodos de reduccion de la plata o por sus modificasiones. *Trab. del Lab. de Invest. de Biol. Univer. de Madrid*, **9**, 161.

— 1913*a*. Notas sobre la estructura y funciones de la neuroglia y en particular de la neuroglia de la corteza cerebral humana. *Ibid.*, **11**, 186.

1913*b*. Histologisches über Gefässverodung und über Erweichung in der Hirnrinde. *Ibid.*, **11**, 19.

— 1915. De l'évolution de la névroglie et spécialement de ses relations avec l'appareil vasculaire. *Ibid.*, **13**, 169.

— 1918. On the evolution of the neuroglia and specially their relations to the vascular apparatus. *J. Nerv. & Mental Dis.*, **48**, 333.

Alzheimer, A. 1910. Beiträge zur Kenntnis der pathologischen Neuroglia und ihrer Beziehungen zu den Abbauvorgangen im Nervergewebe. *Hist. u. Histopath. Arb. Nissl-Alzheimer*, **3**, 401.

Andriezen, W. 1893*a*. The neuroglia elements in the human brain. *Brit. Med. J.* **2**, 227.

——— 1893*b*. On a system of fiber-cells surrounding the blood-vessels of the brain of man and mammals and its physiological significance. *Intern. Monstsschr. f. Anat. u. Physiol.*, **10**, 533.

Aschoff, P. 1924. Reticulo-endothelial system. In *Lectures on Pathology*. New York: Paul B. Hoeber, Inc.

Bailey, P., and Cushing H. 1926. *A classification of the tumors of the glioma group on a histogenetic basis with a correlated study of prognosis.* Philadelphia: Lippincott.

Bailey, P., and Hiller, G. 1924. The interstitial tissues of the central nervous system, a review. *J. Nerv. & Ment. Dis.*, **59**, 337.

Barker, L. 1899. *The nervous system and its constituent neurones.* New York: D. Appleton & Co.

Boll, G. 1874. Die Histologie und Histogenese der nervosen Zentralorgan. *Arch. f. Psych.*, **4**, 1.

Buscaino, V. 1913. Sulla genesi e sul significato delle cellule ameboidi. *Rev. di Pat. nerv. e ment.*, **18**, 360.

Cajal, S. Ramóny. 1890. Sur l'origine et les ramifications des fibres nerveuses de la moelle embryonnaire. *Anat. Anz.*, **5**, 111.

—— 1909 and 1911. *Histologie du système nerveux de l'homme et des vertébrés.* Paris.

—— 1913*a*. Sobre un nuevo proceder de impregnación de la neuroglia y sus resultados en los centros nerviosos del hombre y animales. *Trab. del Lab. de Inv. Biol. de la Univ. de Madrid*, **11**, 219.

—— 1913*b*. Contribución al conocimiento de la neuroglia del cerebro humano. *Ibid.*, **11**, 255.

—— 1916. El proceder del oro-sublimado para la coloración de la neuroglia. *Ibid.*, **14**, 155.

—— 1920*a*. Una modificación del método de Bielschowsky para la impregnación de la neuroglia común y mesoglia y algunos consejos acerca de la tecnica del oro-sublimado. *Ibid.*, **18**, 129.

—— 1920*b*. Algunas consideraciones sobre la mesoglia de Robertson y Del Rio-Hortega. *Ibid.*, **18**, 109.

Castro, F. de. 1920. Estudios sobre la neuroglia de la corteza cerebral del hombre y de los animales. *Ibid.*, **18**, 1.

Cerletti, U. 1903. Sulla neuronofagia e sopra alcuni rapporti normali e patologici fra elementi nervosi ed elementi non nervosi. *Ann. d. Inst. Psichiatrico d. R. Univ. d. Roma*, 91.

Cone, W. 1925. A case of anterior poliomyelitis as studied by the newer staining methods. *Proc. N. Y. Pathol. Soc.*, N. S. **25**, Nos. 1–5, 78.

Da Fano, C. 1906. Osservazioni sulla fine struttura della nevroglia. *Lab. anat. norm. d. R. Univ. Roma e altri lab. biol.*, **12**, 1.

Del Rio-Hortega, P. 1916*a*. Estructura fibrilar del protoplasma neuróglico y origine de las gliofibrillas. *Trab. del Lab. de Inv. Biol. de la Univ. de Madrid*, **14**, 269.

—— 1916*b*. Contribution à l'etude de l'histopathologie de la nevroglie. Les variations dans le ramollissement cerebral. *Ibid.*, **14**, 1.

—— 1916*c*. Gliosomas y gliofibrillas. *Bol. d. l. Soc. Esp. d. Biol.*, 42.

—— 1917. Alteraciones de la neuroglia en la intoxicacion por pilocarpina. *Laboratorio, Rev. de Cienc. Biol. y Med. Esp., Barcelona.*

—— 1918. Sobre la verdadera significacion de las celulas neuroglicas llamadas amiboides. *Bol. de la Soc. Esp. de Biol.*, **8**, 229.

—— 1919. El "tercer elemento" de los centros nerviosos. I. La microglia en estado normal. II. Intervencion de la microglia en los procesos patologicos. III. Naturaleza probable de la microglia. *Ibid.*, **9**, 69.

—— 1920. La microglia y su transformación en células en bastoncito y cuerpos granulo-adiposos. *Archiv. de Neurobiologia;* also *Trabajos del Lab. de Inv. Biolog. de Madrid*, **18**, 37.

Del Rio-Hortega, P. 1921*a*. Histogenesis y evolucion normal; exodo y distribution regional de la microglia. *Mem. Real. Soc. Esp. d. Hist. Nat.*, **11**, 213.

——— 1921*b*. Estudios sobre la neuroglia. La glia de escasas radiciones (oligodendroglia). *Bol. de la Real. Soc. Esp. d. Hist. Nat.*, January.

——— 1922. Son homologables la glia de escasas radiacion es y la celula de Schwann? *Bol. de la Soc. Esp. de Biol.*, 10.

Del Rio-Hortega, P., and Jimenez de Asua, F. 1921. Sobre la fagocitosis en los tumores y en otros procesos patológicos. *Archiv. de Cardiol. y Hematol.*, **2**, 161.

Del Rio-Hortega, P., and Penfield, W. 1926. Cerebral cicatrix. The reaction of neuroglia to brain wounds. *Brain*, to appear shortly.

Deiters, O. 1865. *Untersuchungen über Gehirn und Rückenmark des Menschen und der Säugetbiere*. Braunschweig.

Eisath, G. 1906. Über normale und pathologische Histologie der menschlichen Neuroglia. *Monatsschr. f. Psych. u. Neur.*, **20**, 1, 139, 240.

Fieandt, H. 1910. Eine neue methode zur Darstellung des Gliagewebes nebst Beiträgen zur Kenntnis des Baues und der Anordnung der Neuroglia des Hundeshirns. *Archiv. f. mikr. Anat.*, **76**, 125.

Ganz, A. 1923. Del Rio-Hortega's Derde Element in het centrale zenuwstelsel. *Nederland Tijdschrift voor Geneeskunde*, **67**, (2), 1024.

Golgi, C. 1885–86. Sulla fina anatomia della sistema nervoso. See *Opera Omnia*. Milan, 1903.

Guillery, H. 1923. Entwickelungsgeschichliche Untersuchungen als Beitrag zur Frage der Encephalitis interstialis neonatorum (Virchow). *Zeit. f. Neurol. u. Psychiat.*, **84**, 205.

Hardesty, I. 1904. On the development and nature of the neuroglia. *Am. J. Anat.*, **3**, 229.

——— 1905. On the occurrence of sheath cells and the nature of the axone sheaths in the central nervous system. *Ibid.*, **4**, 329.

Havet, J. 1916. Contribution à l'étude de la névroglie des invertebrés. *Trab. del Lab. de Inv. Biol. de la Univ. de Madrid*, **14**, 36.

Held, H. 1903. Über den Bau der Neuroglia und über die Wand der Lymphgefässe in Haut und Schleimhaut. *Abhandl. d. mathem. physikal. Klasse d. kgl. Sachs. Gesellsch. d. Wissensch.*, 28.

——— 1909. Über die Neuroglia marginalis der menschlichen Grosshirnrinde. *Monatschr. f. Psychiatr. u. Neur.* **26**, 360.

His, W. 1889. Die Neuroblasten und deren Entstehung im embryonalen Mark. *Arch. f. Anat. u. Physiol.*, 249.

——— 1901. Das Princip der organbildenden Keimbezirker und die Verwandschaftendder Gewebe. *Arch. f. Anat. u. Entw.*, 307.

Holzer, W. 1923. Über die Bestandteile des Heldschen Gliasyncitiums. *Zeit. f. d. ges. Neur. u. Psychiat.*, **87**, 167.

Huber, G. 1903. *Studies on neuroglia tissue*. Contributions to Medical Research. Dedicated to V. C. Vaughan. Ann Arbor, 578.

Ingleby, H. 1925. Note on the termination of the podic (perivascular foot) of fibrous neuroglia cells. *J. Roy. Micr. Soc.*, 423.

Kappers, A. 1920. *Die vergleichende Anatomie des Nerven systems des Wirbeltiere und des Menschen*. Haarlem, **2**, (1), 48.

Kölliker, A. 1890. Zur feineren Anatomie des central-Nervensystem. 2nd Beitr.; das Rückenmark. *Zeit. f. wiss. Zool.*, 51.

——— 1893. *Handbuch der Gewebelehre des Menschen*. Ed. 2.

Lenhossék, M. v. 1891. Zur Kenntniss der Neuroglia des menschlichen Ruckenmarkes. *Verhandl. d. anat. Gesellsch. 5th Versamml. Erganz. Anat. Anz.*, **6**, 193.

Lenhossék, M. v. 1895. *Bau des Nervensystems.* Berlin.

Marinesco, G. 1909. *La cellule nerveuse.* Paris: Doin.

Marinesco, G., and Lupa. 1925. Einige Untersuchungen über die Mikroglia in normalen und pathologischen Zustand. *Zentralblatt f. d. ges. Neur. u. Psychiat.*, **41**, 137.

Marinesco, G., and Minea, 1925. Die Kultur des Glia Gewebes der Grosshirnrinde in vitro Angaben zur Bildung und Funktion der amöboiden Zellen. *Zeit. f. d. ges. Neur. u. Psychiat.*, **41**, 137.

Metchnikoff, E. 1913. *Handbuch der pathogenen Mikroorganismen, Kalle u. Wassermann.* Jena. **2**, 654.

Metz, A., and Spatz, H. 1924. Die Hortegaschen Zellen (= das sogenannte "dritte Element") und über ihre funktionelle Bedeutung. *Zeit. f. d. ges. Neur. u. Psych.*, **89**, 138.

Nageotte. 1920. Phenomènes de sécretion dans le protoplasma des cellules névroglique de la substance grisse. *Compt. rend. de la Soc. de Biol.*, **68**, 1068.

Nissl, F. 1904. Zur Histopathologie der paralytischen Rindenerkrankung. *Histologische u. histopathologische Arb. Nissl-Alzheimer*, **1**, 315.

Penfield, W. 1924. Oligodendroglia and its relation to classical neuroglia. *Brain*, **47**, 430.

——— 1925*a*. Microglia and the process of phagocytosis in gliomas. *Am. J. Pathol.*, **1**, 77.

——— 1925*b*. Phagocytic activity of microglia in the central nervous system. *Proc. N. Y. Pathol. Soc.*, N. S., **25**, Nos. 1–5.

Penfield, W., and Cone, W. 1926*a*. A specific type of neuroglia change, acute swelling of oligodendroglia. *Arch. Neur. & Psychiat.*, **16**, 130.

——— 1926*b*. The acute regressive changes of neuroglia. *J. f. Psychol. and Neurolog.*, **34**, 204.

Robertson, W. 1897. The normal histology and pathology of the neuroglia. *J. Mental Sci.*, **43**, 733.

——— 1899. On a new method of obtaining a black reaction in certain tissue-elements of the central nervous system (platinum method). *Scottish Med. & Surg. J.*, **4**, 23.

——— 1900*a*. A microscopic demonstration of the normal and pathological histology of mesoglia cells. *J. Mental Sci.*, 724.

——— 1900*b*. *A text-book of pathology in mental diseases.* Edinburgh: Clay.

Rosental, S. 1913. Experimentelle Studien über amöboide Umwandlung der Neuroglia. *Histo. u. Histopath. Arb. Nissl-Alzheimer*, **6**, 69.

Rubaschkin, W. 1904. Studien über Neuroglia. *Archiv. f. mikr. Anat.*, **46**, 575.

Schaper, A. 1897. Die frühesten Differenzirungsvorgänge im central Nervensystem. *Arch. f. Entwickelungsmechanik*, **5**, 81.

Spielmeyer, W. 1922. *Histopathologie des Nervensystems.* Berlin.

Streeter, G. 1912. Development of central nervous system. In *Manual of Human Embryology*, Keibel and Mall. Phila. **2**, 1.

Strong, O. 1925. In *Bailey's Histology*. New York.

Virchow, R. 1846. Über das granulirte Ansehen der Wandungen der Gehirnventrikel. *Allgem. Zeitschr. f. Psychiat.*, **3**, 242.

——— 1867. Kongenitale Encephalitis und Myelitis. *Virchow's Archiv*, **38**, 129.

Weed, L. 1917. An anatomical consideration of the cerebro-spinal fluid. *Anat. Record*, **12**, 461.

Weigert, C. 1895. *Beiträge zur Kenntnis der normalen menschlichen Neuroglia.* Frankfurt.

Wohlwill. 1914. Über amöboid Glia. *Virchow's Archiv*, **216**, 468.

Ziveri, A. 1914. Alcune considerazioni sulla cosidetta neuroglia adendritica (tercer elemento di Cajal). *Ann. di Neuroglia*, **6**, 3.

Section XXXI

THE CYTOLOGY OF THE CEREBROSPINAL PATHWAY

CONTENTS

Section XXXI

	PAGE
I. Cytology of Cerebral Ventricles	1071
II. Roof of Fourth Ventricle	1078
III. Meninges	1080
IV. Development of Meningeal Spaces	1083
V. Subdural Space	1085
VI. Perivascular and Perineuronal Spaces	1086
VII. Cytology of Leptomeninges	1089
VIII. Arachnoid Villi	1095
IX. Cranial and Spinal Nerve Roots	1099
X. Bibliography	1102

Section XXXI

THE CYTOLOGY OF THE CEREBROSPINAL PATHWAY

G. B. WISLOCKI

BY the closure and further differentiation of the neural tube a cavity is formed which undergoes conversion into a series of intercommunicating chambers. At a somewhat later embryonic period the mesenchyme surrounding the cerebrospinal axis differentiates into a series of membranes known as the meninges. The two innermost of these, or leptomeninges, are loosely attached to one another by numerous fine trabeculae which convert the space separating them into a labyrinth of confluent channels. Both the medullary cavities, or ventricles, as well as these extramedullary spaces normally contain a fluid, the cerebrospinal liquid. The nature of this fluid, its source, the possibility of its circulation in the spaces in and about the brain and cord, and its possible mode of absorption have excited the interest of anatomists, physiologists and pathologists alike during the last century. The earlier workers in this field dealt with the subject, for the most part, in a purely morphological way, often drawing quite unwarranted and speculative conclusions as to the functional rôle of the structures in question. But during the last twenty years in conformity with the growing demand for close correlation of function with morphology by adequately controlled experimental study, a series of investigations has contributed to place this subject upon a sound basis.

The cytology of the cerebrospinal pathway has interested the anatomist ever since the discovery of the cell, and an enormous descriptive literature has grown up on this subject. It would be useless and unprofitable to attempt to give within the scope of a single section, an exhaustive account of the earlier observations. In this review only such of the cytological work as is deemed essential for the development of our modern conception of the cerebrospinal pathway from a functional standpoint will be discussed. This conception has been achieved largely by the experimental work of the past two decades, through the combined use of anatomical and physiological methods.

I. THE CYTOLOGY OF THE CEREBRAL VENTRICLES

The evidence, though not absolute, favors the origin of the major portion of the cerebrospinal fluid from the chorioid plexuses of the cerebral ventricles. This view takes its inception from the observations of Faivre (1853) and Luschka (1855). Previously, it had been held by Haller (1757) and by Magendie (1825) that the fluid was derived from the leptomeninges.

LANE LIBRARY. STANFORD UNIVERSITY

Faivre and Luschka were the first investigators to subject the chorioid plexus to a careful histological examination. They were so much impressed by the gland-like character of these villous structures, with their covering of epithelial cells which contained hyaline-like droplets and other inclusions, that they hypothesized a secretory activity on the part of the plexuses. This view was tacitly accepted for many years by subsequent writers without contributing any substantiating evidence other than equivalent histological findings. Thus Findlay (1899) described the presence of pigment granules in the normal epithelium of the chorioid plexuses; while Studnicka (1900) in an exhaustive series of preparations of vertebrate brains described the presence of secretion granules in the plexuses and various other portions of the ependymal lining of the ventricles. Similarly, Galeotti (189–), Pettit and Girard (1902), Loeper (1904), Schläpfer (1905), Francini (1907), Engel (1909), Yoshimura (1910), Hworostuchin (1911) and Pellizzi (1911) have described the histology of the chorioid plexuses, in part reaffirming, in part contributing new details, but in the main failing to advance materially beyond the surmise of Faivre and Luschka. However, these studies have given us a careful morphological survey of the chorioid plexuses.

It is necessary in an inquiry into the nature and origin of the chorioid plexuses to consider the entire walls of the cerebral ventricles, because the plexuses are nothing more than differentiated portions of these surfaces. Early in embryonic life certain of the spongioblasts of the neural tube arrange themselves in an epithelial layer at the surface of the medullary cavity. These cells become the ependymal lining of the ventricles and they acquire certain characteristic cytological features. A large descriptive literature has grown up during the last century concerning them.

The first mention of the epithelial character of the lining of the medullary tube, as well as of the presence of cilia upon the cells, was made in 1836 by Purkinje in collaboration with Valentin. Their observations were corroborated and extended by Hannover (1844) and by Kölliker (1854) and the latter was able by direct observation to ascertain the connection of the cilia with the cells. A comprehensive description of the ependyma of the spinal cord with the staining methods then available was contributed by Stilling in 1859, while Mauthner in 1861 succeeded in tracing what he believed to be processes from the epithelial cells through the entire thickness of the dorsal septum of the spinal cord of the trout. Reissner (1864) and Stieda (1869, 1875) verified this finding in numerous animals, but the observation passed practically unnoticed. Fromman (1864) investigated the manner of obliteration of the central canal of the spinal cord of man; Mierzejewski (1872) described the character of the ependyma in different regions of the human brain; Eichhorst (1875) remarked upon the ependyma in the central nervous system of the human embryo; Renaut (1882) described the microscopic anatomy of the ependyma in Petromyzon; Klaussner (1883) in Proteus; and Löwe (1883) studied the ependyma of the human spinal cord. None of these investigators contributed more than additional details, but with the introduction of the important neurological techniques of Golgi and Cajal much new information concerning the morphology of the ependymal cell was soon obtained. Golgi himself, in 1885, with the silver technique succeeded in tracing processes of the ependymal cells through the spinal cord of the chick embryo to

the pia mater, thereby corroborating and fully establishing the earlier findings of Mauthner and Stieda and awakening renewed interest in the subject. His observations were verified and extended by Nansen (1887), Ramon y Cajal (1889, 1890), Kölliker (1890), Retzius (1893), Kolster (1898), Eurich (1898), Bonne (1899) and others using a variety of material. The newly devised technique of Weigert and the iron hematoxylin method were employed as well for study of the ependyma.

It is not purposed in this review to discuss the embryology of the ependyma. It will suffice to say that it is a question which is intimately bound up with the entire subject of the differentiation of the neurogliar tissue and one that would take us too far outside the province of our discussion. For reference to the earlier work on this subject mention is made of the papers of His (1889) and Schaper (1894, 1897).

For a morphological description of the ependyma using the whole range of modern histological technique on a large comparative series of vertebrate animals, it is necessary to turn to the paper of Studnicka (1900). This study impresses one by the wide latitude in the morphology of the cells in different animals, at different ages and in different regions of the central canal and ventricles. No general description will do complete justice to the wealth of structure encountered there. The cells range from low, endothelial-like forms, to cuboidal ones, to columnar ones with, and without, cilia and cell processes. The ependyma is formed of a single row of cells, erroneously thought by some to be in places many-layered. Cilia are present with great regularity in lower vertebrate forms and during fetal life in mammalia. Peripheral processes extend from the cell bodies through the cerebral wall to end with terminal expansions as the external limiting membrane in the pia (Golgi, Nansen, Ramon y Cajal, Retzius, Studnicka). These fibers may possess numerous branches, but do not anastomose with one another. In the course of development of the nervous system of the higher vertebrates and man particularly, the peripheral ends of the fibers undergo degeneration and terminate without reaching the periphery of the brain. (Ramon y Cajal, Studnicka.) In the literature a variety of spaces are described between and beneath the cell bodies, termed lymph spaces, which in the light of our modern conception of the brain are nothing more than parts of the system of intercommunicating tissue spaces recognized as occurring everywhere in the tissue of the brain. Intercellular bridges are said to occur between the cell bodies of adjacent ependymal cells in some forms (Studnicka). On their free surfaces the ependymal cells possess cuticular borders forming an external limiting membrane which is considered to be a part of the exoplasm of the cells. The cuticula is of variable distinctness and structure in different regions and it exhibits perforations through which the free ends of the cilia project into the ventricles.

The cilia themselves, first described by Purkinje and Valentin and subsequently observed by numerous investigators, have been discussed more particularly by Lenhossek (1891), Retzius (1893), Prenant (1894) and Studnicka (1900). In the very earliest embryonic stages they are absent and according to His, in the human, they do not begin to appear upon the spongioblasts until about the end of the fifth week. The cilia occur in all the lower vertebrate forms, but in the higher animals their existence is transient, so that in man, according to Studnicka, they disappear in the further course of development from all except restricted regions of the ependyma, as for instance, in the adult, on a few cells of the rhomboid fossa and of the aqueduct of Sylvius. Their number, size, length and distribution upon the cells vary, although generally several or a small bundle of them arise near the center of the cell surface. Basal granules occur at the points of attachment of the cilia to the cell.

The chorioid plexuses arise from the membranous portions of the roof of the forebrain and hindbrain, at first as a series of buds or low folds, but

becoming increasingly more folded as growth continues. These folds consist of a richly vascularized connective tissue core, derived from the periaxial mesenchyme, and a covering of epithelial cells, the lamina epithelialis. The latter consists of but a single layer of cells which constitute the entire thickness of the neural wall in those portions of the membranous ependyma destined to become the plicae chorioideae. Hence the lamina epithelialis represents a modified part of the ependymal lining of the ventricles and blends with the latter by a gradual transition along the lines of origin of the tela chorioidea.

Histologically, the chorioid plexuses exhibit an epithelium consisting of a single layer (Studnicka, 1900; Meek, 190-) of cuboidal cells whose sides abut upon one another without demonstrable intervention of intercellular spaces or substance. In higher vertebrates (Milian, 1904; Pettit and Girard, 1902; Meek, 190-), throughout embryonic life, cilia are often present upon their free surfaces, but during the further course of development every vestige of them disappears. A delicate cuticular or brush border may be demonstrated in stained specimens. The bases of the cells lack ependymal processes and constitute the external limiting membrane by which the cells are attached directly to the connective tissue stroma of the chorioidal folds. The mesodermal stroma possesses a rich blood supply consisting of plexiform capillaries of wide caliber. Luschka first demonstrated the abundant vascularity of these structures by injecting the blood vessels.

In spite of the fact that ganglion cells and neuroglia have been described as occurring beneath the ependyma of the plexuses, it is now generally conceded that the plexuses do not possess such elements (Studnicka, 1900). Nevertheless Findlay (1899) describes nerves in the neighborhood of the blood vessels in the plexus of man and of the calf, but they must be considered as vasomotor in nature. In the stroma of the chorioidal folds amongst the blood vessels, one finds numerous wandering connective tissue cells capable of phagocytizing particulate matter as has been amply demonstrated by Goldmann (1913) in a series of vitally stained rats and mice. Kolmer (1921) has made the curious observation that, in the vitally stained frog, these so-called macrophages wander out not infrequently between the epithelial cells and may be found clinging to the free surface of the plexuses. What rôle they play in this manner cannot be surmised. Another curious property of the chorioid plexuses is the occurrence of glycogen within the epithelial cells; abundant during fetal life (Goldmann, 1913; Weed, 1917), the reaction vanishes soon after birth. It is of course well known that glycogen enjoys a much wider distribution in the cells of the fetal organism than of the adult; but the significance of this difference is unknown.

As has been outlined above, the earlier investigators of the ependyma conjectured from the gland-like appearance of the chorioid plexuses that they subserve the function of elaborating the cerebrospinal fluid. The evi-

dence, however, was scarcely more than circumstantial. Further proof of a more convincing nature utilizing experimental methods has since then been obtained. Thus Cappelletti (1900) reported that ether and pilocarpine increased the flow of cerebrospinal fluid from a cannula inserted into the cisterna magna while atropine and hyoscyamine diminished it. Pettit and Girard (1902) undertook similar experiments, but at the same time examined the chorioid plexuses histologically. After administration of muscarine, pilocarpine, ether and theobromine they found that the chorioidal epithelium was profoundly changed, so that the cells became doubled in height. This transformation of the cells was associated with characteristic cytoplasmic changes consisting of the formation of a densely granular basilar zone and a clear apical zone. Meek (190-), repeated Pettit and Girard's experiments and corroborated their observations. The facts that the flow of cerebrospinal fluid is increased by injections of drugs that usually stimulate secretion in epithelial cells and that the chorioidal cells show secretory changes justify, he believes, the conclusion that the fluid is secreted by the chorioid plexuses.

Evidence from pathological and embryological standpoints contributes other strong corroborative support for this conception. Consideration of internal obstructive hydrocephalus speaks unequivocally for the intraventricular source of the fluid; and the concomitant hypertrophy of the chorioid plexuses, as observed by Claisse and Levy (1897) in such a brain, points strongly towards the plexuses as a source of the ventricular fluid. The most indubitable evidence for this hypothesis are the experiments of Dandy (1919) in which he produced hydrocephalus of one lateral ventricle in the dog by plugging the foramen of Monro, but demonstrated that, if at the time of occlusion of the foramen, the chorioid plexus of the chamber was extirpated surgically, hydrocephalus failed to develop. Mention should also be made of the substantiating observation by Cushing (1914) who noticed an exudation of fluid from the surface of the plexuses exposed during a surgical exploration. From the embryological side Weed (1917) points out that in the pig embryo the first extraventricular escape of the fluid is coincident with the initial tufting and histological differentiation of the ependymal cells destined to form the chorioid plexuses.

The most convincing proof of this conception, however, is contributed by Weed (1923) in a recent study of the effects of hypotonic solutions upon the cell morphology of the plexuses. He administered intravenous injections of hypotonic solutions in large amount to dogs and found that the increase in the pressure of the cerebrospinal fluid and in the volume of the brain, brought about by this means, were associated with definite morphological changes in the epithelial cells of the chorioid plexuses. The cell changes were in the nature of an increased volume of the cells with the formation of a clear vacuolar zone in the distal portion of the cell. Weed states that the

findings may be considered as affording morphological evidence of the passage of water into the cerebrospinal fluid and into the nervous tissues under such experimental conditions. Moreover, in a recent review Weed (1922) gives a critical discussion of the relative value of the evidence in favor of the view that the chorioid plexuses are the elaborators of the major portion of the cerebrospinal fluid. He points out that the evidence from the histological, pharmacological, pathological and embryological standpoints inclines one to acceptance of the hypothesis. However, he observes that it does not seem justifiable to accept the evidence from any one standpoint as conclusive, for many of the observations are corroborative only. In discussing the objections raised by Becht (1920) to the acceptance of this hypothesis, Weed states clearly that while many of the experimental findings, when viewed as isolated observations, may be explained by other hypotheses, the great mass of data cannot be interpretated on any other hypothesis as satisfactorily.

The cerebrospinal fluid is a liquid differing from other body fluids in its chemical composition and containing small quantities of inorganic salt, protein and dextrose. It cannot, therefore, be a direct transudate from the blood plasma, but must, in its derivation from the latter, be modified by the intermediary action of a selective semi-permeable membrane. The cells of the chorioid plexus constitute such a membrane which serves as the mechanism interposed between blood stream and ventricles. As examples of such protection one may recall the difficulty which is experienced in attempting to treat disease of the central nervous system by introducing a therapeutic agent via the blood stream. Another striking example may be seen in the vitally stained animal. Goldmann (1913) observed that after repeated intravenous injections of the vital dye, pyrrhol blue, into a rat or mouse all of the tissues of the body became deeply colored with the exception of the central nervous system. In the brain the chorioid plexuses alone had become deeply stained: the reason for this was that the dye had accumulated in dust-like granules within the chorioidal epithelial cells, being evidently passed from the blood stream to these cells, where it was retained. Since none of the dye could be recovered from the cerebrospinal fluid, the observation of Goldmann must be interpreted as proving that towards the ultramicroscopic sols of this class of dyes the chorioidal epithelium is impermeable. It should be mentioned that the hypophysis, and the outer meningeal coat, the dura, and, as observed subsequently by Wislocki and Putnam, the area postrema are the only other portions of the central nervous system besides the chorioid plexuses which become stained vitally after intravenous injections.

Attention has constantly been focussed upon the chorioid plexuses as the major ventricular source of the cerebrospinal fluid. It has been suggested, however, that the entire ependyma lining the neural tube may par-

ticipate to some extent in its elaboration. Studnicka (1900), in studying the brains of certain vertebrates in fixed and stained material, saw vacuoles and droplets upon the apical surfaces of ependymal cells in some instances, which he interpreted to indicate secretory activity. On the whole, however, the relatively low ependyma prevailing in the ventricular walls in the adult and the relative paucity of blood vessels beneath it do not suggest a secretory function. Moreover, the experimental evidence that exists regarding this question points rather to the contrary, for Wislocki and Putnam (1921) and Nañagas (1921) have shown that after experimentally establishing an hydrocephalus in kittens, absorption of fluid takes place through the ventricular walls into neighboring veins. Dandy and Blackfan (1913,1917) have recorded similar findings with phenolsulphonephthalein in hydrocephalic human beings. Nañagas has shown that absorption occurs as well from the normal ventricles under the influence of intravenous injection of hypertonic salt solution. Neither Wislocki and Putnam, nor Nañagas, have seen any evidence of absorption by the chorioidal ependyma, an observation which further substantiates the probable secretory nature of that structure.

There is some evidence, however, that in addition to the chorioid plexuses, which are undoubtedly the major intraventricular source, the cerebrospinal fluid receives accessions from at least one other portion of the ventricular wall. Wislocki and Putnam (1924) have drawn attention to the area postrema of the calamus scriptorius of the fourth ventricle. In mammals this area consists of two mounds of exceedingly vascular tissue, projecting into the lumen of the ventricle, which fuse caudally to form the roof of the central canal of the cord. In vitally stained animals these areas become deeply colored in sharp contrast to the unstained brain and cord. Likewise, on injecting a balanced solution of iron ammonium citrate and potassium ferrocyanide into the blood stream of the living animal, subsequently killing it and precipitating the reagents as Prussian blue, it can be seen that the experimental salts have diffused into the tissue of the area postrema. The solutions may be traced in these areas from the capillaries, through the perivascular tissue spaces, to the delicate, endothelium-like ependyma which the salts finally penetrate to reach the ventricle. The remainder of the ventricular wall does not react in this manner. The observers postulate for the area postrema a region where the cerebrospinal fluid receives an addendum of fluid as a direct transudate from the blood stream, without the ependyma exercising a regulatory or secretory control as it does in the chorioid plexus.

One cannot pass over this subject without mentioning the possibility, first suggested by Herring (1908), that the posterior lobe of the hypophysis pours its secretion into the third ventricle via the infundibulum. He thought that he could trace the migration of hyaline bodies between the ependymal cells into the cavity of the third ventricle. Cushing and Goetsch (1910) corroborated Herring's histological findings, but Edinger

(1911) from injection experiments and study of sections concluded that, although the hyaline bodies migrate along interneuroglial pathways towards the region of the tuber cinereum, they do not enter the ventricle. Maurer and Lewis (1922) and Hines (1923), on the other hand, investigating the cytology of the cells of the posterior lobe and pars intermedia, failed to find any histological evidence in favor of the passage of secretion products from the hypophysis into the cerebrospinal fluid.

The question has been discussed principally, however, on the basis of the presence of pharmacologically active substances, identical with posterior lobe hormones in the cerebrospinal fluid. Cushing and Goetsch (1910) described the presence of pressor substance in the cerebrospinal fluid, giving reactions similar to those obtained from posterior lobe extracts. The observations of Carlson and Martin (1911), Houssay (1918) and Jacobson (1920) made it appear doubtful that the posterior lobe gives off its secretion into the cerebrospinal fluid. However, Dixon (1923) and Dixon and Marshall (1924) have recently demonstrated the presence of a markedly oxytocic substance in the cerebrospinal fluid of the dog. Trendelenburg (1924) and Miura (1925) working contemporaneously, have made the same observation and have shown, moreover, that after extirpation of the hypophysis the oxytocic substance disappears from the cerebrospinal fluid. (See Cushing, 1925, and Geiling, 1926.)

II. THE ROOF OF THE FOURTH VENTRICLE

The roof of the fourth ventricle deserves special attention because the ependyma in that region is especially modified to allow the cerebrospinal fluid to escape from the ventricles into the subarachnoid space. The prevailing view holds that the membranous roof of the rhombencephalon is perforated by a median opening, the foramen of Magendie (1846) and by two lateral clefts, the foramina of Luschka (1855). Doubt has been expressed, however, as to the actual existence of these apertures, some observers considering them merely as artefacts produced by dissection and preparation of sections or by post-mortem changes, and believing that, in the adult as in the embryo, the velum is composed of an unbroken membrane. The difficulty of deciding this question definitely is obvious. The morphological observations of Retzius (1896) and Blake (1900) and Wilson (1906) support the idea of open communications. Blake more particularly, in a developmental study of the region, describes the formation of the foramen in the inferior velum by a shearing off at the base of a finger-like evagination of the rhombic roof.

The roof of the rhombencephalon, forming as it does part of the wall of the neural tube, is unbroken at least during its earliest development. Weed (1917) has made a comprehensive morphological and functional study of this region during the embryonic period in the pig and in man. He devised a method of replacing the fluid in the medullary canal of the living embryo by a foreign solution. Potassium ferrocyanide and iron ammonium citrate, prepared in isotonic solutions, have proved invaluable in this and others of his studies in tracing the fluid pathways in and about the central nervous system. These salts form crystalloidal solutions which are non-toxic to living tissue and are readily diffusible. Moreover, the salts can be precipi-

tated in the tissue at the termination of the experiment as Prussian blue by the addition of weak mineral acid to the fixing solution. Tissues prepared by this method can be studied in sections or by clearing. Besides the Prussian blue reagents, suspensions of particulate matter in the form of India ink or of suspended lampblack have been valuable supplementary agents to the true solutions in the differentiation of anatomical and functional pathways.

In pig embryos, according to Weed, in which the intramedullary fluid has been replaced, the foreign solution is retained in the ventricles without periaxial spread until a stage of 14 mm. crown-rump length is reached. In 9 mm. embryos there is no characteristic distribution of the Prussian blue in the ventricles, but in stages of 13 mm. a dense blue staining of an oval area in the superior portion of the membranous rhombic roof can be discerned. Histologically, the ependymal cells composing this areas differ from the ependyma of the surrounding ventricular wall. The cells possess relatively irregular cell borders and spindle-like, elongated, faintly stained nuclei in contrast to the usual pycnotic nuclei of the undifferentiated ependyma. This hitherto undescribed area is termed by Weed the area membranacea superior. In living pig embryos of 14 mm. or older, a slight extravasation of the Prussian blue reagents takes place through this area into the perimedullary mesenchyme. This extravasation remains localized at first to a small conical area of the primitive meninx.

The time of this escape of the first fluid from the ventricle into the periaxial tissue coincides with the first differentiation of the chorioid plexuses. At the stage of 14 mm. in the pig embryo the ependyma of the roof of the fourth ventricle thickens along the transverse invagination, the plica chorioidea, from which a series of small ependymal tufts arise. The chorioid plexuses of the third and lateral ventricles develop in a similar manner at a slightly later stage, in 19 mm. embryos. It is probably no mere coincidence that the development of the plexuses and the perimedullary spread of fluid occur simultaneously; the occurrence is in keeping with the most plausible conception of the origin of the cerebrospinal fluid.

At a slightly later stage, in the 18 mm. embryo and over, and pari passu with the progressive development of the chorioid plexus of the fourth ventricle and the appearance of chorioidal tufts in the forebrain ventricles, a second area of escape of the Prussian blue reagents appears in the roof plate. This, the area membranacea inferior, occupies the inferior half of the roof and is separated from the superior membranous area by the zone of developing chorioid plexus. Likewise, the inferior area exhibits histologically a rapid differentiation of the ependyma of the inferior chorioidal velum, consisting in a characteristic enlargement of the entire cells and their nuclei. With the establishment and functional employment of the two membranous areas, the peribulbar spread of the replaced fluid increases rapidly.

The two membranous areas in the roof of the fourth ventricle differentiate from the ependyma to subserve specific functional purposes. The superior membranous area attains the height of its activity between the 18 to 20 mm. stage, but thereafter slowly undergoes retrogression, giving way to structural rearrangements in that region due to the growth of the cerebellum and the extension of the chorioid plexuses, until complete obliteration overtakes it. Meanwhile, the inferior membranous area increases steadily in circumference and functional importance. Its ultimate fate involves the question of whether or not it persists as a complete membrane throughout life, or finally ruptures, giving rise to an open communication between ventricle and subarachnoid space.

In a series of sections of human embryos, too, Weed has traced a similar differentiation of the ependyma of the rhomboid roof with a subsequent obliteration of the superior and persistence of the inferior area. In the human, study of the material speaks in favor of the ultimate formation of a foramen by the shearing off at its base, as Blake first described it, of an evagination of the inferior velum. Final proof, however, that either this foramen or the two lateral ones —those of Luschka —actually exist is still lacking.

III. THE MENINGES

The meninges of mammals consist of three envelopes of mesodermal origin enclosing the brain and spinal cord. The origin of these membranes in a comparative series of vertebrates has been made the subject of study and hypothesis by Sterzi (1900, 1902). In Amphioxus he describes the existence of an undifferentiated sheath of mesoderm surrounding the central nervous system. In cyclostomes, elasmobranchs and teleosts he traces the separation of this sheath into two membranes, the outer, or endorrhachis, serving as the perichondral or periosteal lining of the vertebral canal, the inner forming a vascular membrane, the "primitive meninx" closely investing the brain and cord. The latter alone, according to Sterzi, is concerned in the formation of the meninges. The two membranes are separated by a perimeningeal space containing mucous secretion or fat cells.

In the urodeles and progressively in the anura, reptiles and birds, the primitive meninx splits into two membranes, the outermost of which becomes the fibrous dura, separated by the subdural space from the inner or primitive pia mater (secondary meninx). The latter is extremely vascular.

In mammalia the secondary meninx, according to Sterzi, becomes greatly thickened and separates further into two layers of which the inner becomes the definitive pia mater (pia mater secundaria) while the outer becomes the arachnoid. These two layers are connected by a multitude of fine trabeculae, the open meshes of which become the subarachnoid space.

The same hypothesis holds for the formation of the meninges surrounding both cord and brain, except that within the cranium the dura and endorrhachis have fused with the obliteration of the peridural space.

Despite the simplicity of Sterzi's conception much of it will probably be found to be incorrect. Already Hansen-Pruss (1923) has conclusively shown that the meninges of birds may be considered to be essentially similar to the three definitive membranes of mammals. He finds a functional subarachnoid space, containing a true cerebrospinal fluid everywhere about the brain and cord except in the region of the sinus rhomboidalis.

Besides establishing the existence of three meningeal coats and a typically developed pia arachnoid in birds, Hansen-Pruss describes certain peculiarities of the meninges of aves. The most unusual of these is the sinus rhomboidalis, a space at the caudal end of the vertebral canal, which he believes is a portion of the subarachnoid space which has become isolated due to a fusion of the pia and arachnoid rostral to the region of the sinus. The morphology of the sinus rhomboidalis he believes is homologous to that of the subarachnoid space and the pia arachnoid enclosing it, the only difference being that instead of containing cerebrospinal fluid, the sinus encloses a gelatinous material. Furthermore, the meninges of birds are characterized by other peculiarities. The epidural space in the vertebral canal is largely obliterated due to a widespread fusion of the dura with the periosteum. Similarly, the subdural space is frequently interrupted by fusion of the arachnoid to the dura. Recently Kappers (1924) has made a careful morphological study of the sinus rhomboidalis. He finds that the gel occupying the sinus is not located extracellularly within tissue spaces, but is composed of the fluid cytoplasm of large vacuolated cells belonging to the leptomeninges.

Moreover, to any one who has an opportunity to observe the complex and variable meninges of the cartilaginous and bony fishes, the difficulty of applying Sterzi's simple scheme must at once become apparent. This question has been reopened by the recent studies of Kappers (1925) and of Van Gelderen (1925), whose results, however, are as yet confined to too few species to allow phylogenetic comparisons between fish and other vertebrates to be drawn.

The basic morphological and cytological structure of the mammalian meninges is quite adequately understood as the result of a number of investigations of a combined physiological and anatomical nature starting with the great work of Key and Retzius in 1875. The dura mater, or pachymeninx, is a tough fibrous sheath adherent to the bones of the cranium in its cephalic portion, but separated in the vertebral canal from the periosteum of the vertebrae by an epidural cushion of fat. It is separated internally from the arachnoid by a partially interrupted narrow cleft, the subdural space, which is lined by characteristic flattened mesothelial cells. The dura is composed, as illustrated by Key and Retzius, of dense, interwoven bundles of fibrous tissue sparsely dotted with nuclei.

The arachnoid is a delicate continuous sheet of connective tissue which by its outer surface forms the opposite wall of the subdural space. It bridges the sulci of the brain and cord and encloses between it and the pia the cerebrospinal fluid in the subarachnoid space. Fine trabeculae of arachnoidal

tissue traverse this space to attach to the pia mater. The latter is a thin membrane which closely invests the surface of the brain following all of its contours. The pia and arachnoid, variously called the leptomeninges, or pia arachnoid, embrace a series of intercommunicating spaces within their meshes. The membranous and trabecular confines of these spaces are completely lined by pavement-like mesothelial cells.

Key and Retzius were the first to demonstrate the continuity of the subarachnoid spaces about the central nervous system by observing the spread of a colored gelatin mass from the point of injection.

The vascular supply to the brain and cord courses within the connective tissue stroma of the pia arachnoid in the form of rich networks of small arteries and veins. These vessels constantly give off or recieve perpendicular branches which pierce the pia to enter or leave the cortex, or white matter of the cord or medulla, in which they communicate with the capillary beds. The arachnoid membrane is avascular, possessing no capillaries and containing blood vessels only in so far as the small arteries and veins just described received joint coverings of stroma and mesothelial cells from both pia and arachnoid. These vessels are usually described as lying in the pia, but it is obvious that, with the close association by means of trabeculae entered into by the two membranes, such a sharp distinction cannot be made, especially as the majority of the vessels course along the pial surface protruding well into the subarachnoid space and at times actually running in the larger trabeculae traversing that space.

We observe, then, that the brain of mammals is surrounded by three membranes, an outer tough fibrous one, the dura mater, and two inner ones, the leptomeninges which are relatively delicate and are closely associated with one another. Moreover, there are two distinct cavities between them, an outer subdural space and an inner subarachnoid space, both of which are lined by cells partaking of the character of mesothelium by which term we designate in the modern sense cells derived from the middle germ layer forming continuous sheets of pavement-like elements confined to the walls of characteristic body cavities. By this term we separate sharply and obviate confusion with the designations endothelium and epithelium.

The subdural cavity is in reality only a potential space, for its walls are in apposition to one another and normally its lumen contains at most only a film of moisture; whereas the subarachnoid space contains an abundant clear fluid which may be easily tapped by an occipito-atlantoid or lumbar puncture and which continues to flow from the needle during the course of the experiment. The relationship of the meninges and these fluid spaces to the circulation and resorption of the cerebrospinal fluid has been one of the fascinating problems in the study of the central nervous system. Only after prolonged experimentation using the range of modern physiological and anatomical methods have we arrived in large measure at the solution of

some of the questions pertaining to this problem. These it is now our purpose to outline.

IV. THE DEVELOPMENT OF THE MENINGEAL SPACES

That the meninges in mammals arise as a differentiation of the middle germ layer about the neural tube is now fully accepted as established by the work of His (1865) and of Kölliker (1879). According to their view the primitive mesenchyme about the neural axis condenses into two laminae, the outer of which, the dura, becomes intimately associated with the inner surface of the cranium and vertebral canal, while the inner lamina, representing the leptomeninges, surrounds the brain and cord. The outer layer, at first closely affiliated with the periosteum of the bones, separates gradually from the latter in the spinal region, thereby producing the epidural space. A similar loosening and eventually complete separation also occur between the dura and the inner lamina, giving rise to the subdural space. Finally, the inner lamina itself divides partially by a condensation of the mesenchyme of its inner and outer surfaces, giving origin gradually to two membranes, arachnoid and pia, which remain connected with one another, however, by a porous mesenchymal stroma. These pores or tissue spaces finally enlarge and assume the form and character of the subarachnoid spaces.

This much was known when Weed in 1917 approached the question from the combined functional and morphological side. Continuing our account of how, in the embryo pig, the chorioid plexuses begin to differentiate at a time when the Prussian blue solutions, introduced into the living embryo by replacement, appear in two areas of the rhombic velum, Weed observed that the foreign solution first passes through the ependymal roof of the rhombencephalon to penetrate the perimedullary mesenchyme in the 14 mm. embryo. Surrounding the brain before this period the mesenchyme consists of an undifferentiated, loose, syncytial network of cells. At about the time of the initial perimedullary spread of the ventricular fluid a cranial blastemal condensation occurs within the external portion of this mesenchyme and simultaneously a less pronounced though definite cellular thickening occurs in that portion of the mesenchyme in contact with the neural tube. The mesenchyme between these two layers retains its loose syncytial character. The outer zone of condensation is the forerunner of the bone, periosteum, dura and, as we shall see, of the membranous arachnoid. The inner zone immediately about the neural axis is the precursor of the pia, while the loose-meshed mesenchyma between the two is the antecedent of the arachnoid trabeculae and spaces. The mesenchyme in contact with the membraneous areas of the rhombencephalic roof remains loose in character, failing to undergo a pial condensation.

It is into this loose mesenchyme, in contact with the membranous areas of the ependymal roof of the fourth ventricle, that the replaced fluid escapes from the ventricles at the 14 mm. stage and it is shortly after this initial transit that a pronounced change in the character of this loose meshwork is inaugurated. This change is of the nature of a transformation of the small tissue spaces of the loose mesenchyme into larger typical subarachnoid channels. Simultaneously with the rapid growth of the chorioid plexus and the further differentiation of the filtration areas in the roof of the ventricle, this fluid passage into the mesenchyme becomes still more augmented, with the result that more and more tissue spaces dilate and many of the intervening septa break down, giving rise to larger and larger arachnoidal spaces. This reorganization extends gradually farther and farther away from the initial points of entry of the cerebrospinal fluid into the periaxial mesenchyme. Meanwhile, the subarachnoid spaces in the angle between the cerebellum and medulla enlarge so much that they give rise to a characteristic cavernous reservoir in that region, termed the cisterna magna, a name first applied to it by Key and Retzius. The fluid in the subarachnoid spaces at this time is rich in albumen as witnessed by the abundant precipitate within the dilated spaces, a characteristic that distinguishes the cerebrospinal fluid of the embryo from that of the adult.

As this metamorphosis of the loose mesenchyme extends rostrally and caudally, the arachnoidal membrane separates gradually from the external blastemal layer and becomes more sharply defined, while the mesenchymal strands, which do not disintegrate in the opening up of the subarachnoid space, establish themselves as the definitive trabeculae connecting the arachnoidal membrane to the pia. At the same time the mesenchymal cells adjacent to the lumen of the arachnoidal spaces become modified, arranging themselves by flattening out, into a complete cellular investment for the internal surface of the arachnoid membrane and of the trabeculae and the external surface of the pia. At a slightly later period the arachnoid separates in many places completely from the external blastemal condensation, the inner portion of which is destined to become the dura, so that clefts arise between these two layers. A similar transformation of the mesenchyme bordering these subdural clefts occurs, and they too acquire a lining of flattened mesothelial cells. At about the 26 mm. stage the differentiation of the subarachnoid space has reached both the cephalic and caudal extremities of the neural tube as evidenced by the spread of the experimental solution.

In human embryos Weed was able to trace histologically a developmental metamorphosis to all intents and purposes similar to that seen in the pig. It will be observed, however, that there is some discrepancy between the conception of Sterzi, His and Kölliker, concerning the differentiation of the primary and secondary meninges, and Weed's account of an external blastemal condensation, giving rise to dura and arachnoid, separated by a

loose-meshed syncytium from a lesser condensation, giving rise to the pia. The validity of these views must be tested further, more particularly from the standpoint of phylogenesis of the meninges. In a previous section some doubt was expressed as to the correctness of Sterzi's view, especially in the light of a recent study of the bird's meninges by Hansen-Pruss.

Another view concerning the origin of the meninges has been expressed recently by Harvey and Burr (1924). Inasmuch as the nerve sheath cells, which Harrison has shown are derived from the neural crest, are analogous to the leptomeninges in their relationship to the nerve structures which they surround, they investigated the possibility that the pia arachnoid is derived from the neural crest. The dura, on the other hand, they thought probably developed from the mesenchyme. Previous observation by Harvey and co-workers had demonstrated that the repair of the dura after injury differs from that of the leptomeninges, a behavior which seemed to indicate a fundamental histogenic difference between these two structures.

They attempted to verify this hypothesis experimentally by transplanting portions of the neural tube of Amblystoma with and without neural crest cells. In the first series of experiments in which part of the nervous system including neural crest cells was transplanted into the tissue just anterior to the limb, the transplant became completely enclosed by a leptomeninx as well as dura. However, in the second series, in which a portion of neural tissue without neural crest cells was similarly transplanted, the leptomeninx failed to develop whereas an incomplete dural membrane was formed. From these observations they concluded that the leptomeninx arises from neural crest cells, while the dura is probably developed from mesenchyme.

V. THE SUBDURAL SPACE

Anatomically the subdural space communicates in nowise with the subarachnoid space, as was first shown by the specimens of Key and Retzius (1875) in which they demonstrated a complete filling of the subarachnoid space of both cord and brain without simultaneous injection of the subdural space. Their observations disproved the then current belief that the cerebrospinal fluid occupied the subarachnoid space in the spinal cord, but that in the skull the subdural space represented an analogous pathway. These investigators demonstrated, moreover, by the silver impregnation method that the subdural space is lined by a continuous layer of mesothelial cells.

The question concerning the possibility of a physiological exchange of fluid between the two spaces has been more difficult of solution. Quincke (1872) observed, after injection into the subdural space, the passage of cinnabar granules into the subarachnoid space; on the other hand, injections made into the subarachnoid space failed, in keeping with the previous experiments of Key and Retzius, to demonstrate a passage of the

foreign particles in the opposite direction. Quincke's experiments with cinnabar in the living animal as indicating a pathway for fluid, however, are open to criticism, because we know that particulate matter is prone to transportation by migratory cells: Quincke remarked upon the intense phagocytic reaction in the tissue in his experiments. Hill (1896) has been a steadfast proponent of the older view that, by the presence of actual foramina, or by filtration, fluid passes with the greatest ease from one space into the other. Finally, Weed (1914) reaffirmed the position of Key and Retzius by demonstrating in numerous experiments on dogs and cats that the Prussian blue reagents, introduced by replacement under physiological pressures into the subarachnoid space of the cord, spread rapidly to all parts of the cranial subarachnoid space, but never leave the confines of the arachnoid to enter the subdural space. At most, under abnormally high pressures, there may occur some slight discoloration of the arachnoidal mesothelium. Concerning the possibility of physiological passage of fluid in the opposite direction, little can be said beyond affirming the unlikelihood of such an occurrence in the light of our present knowledge of the nature of the subdural space, which at most contains only a film of fluid and resembles very closely a serous cavity.

VI. THE PERIVASCULAR AND PERINEURONAL SPACES

As we have outlined, it is now well established that the subarachnoid space is formed of intercommunicating channels surrounding the brain and cord and filled with cerebrospinal fluid. According to the best conception the major portion of this fluid is intraventricular in origin and gains access to the subarachnoid space through the roof of the fourth ventricle. There is, however, considerable evidence that the cerebrospinal fluid in the subarachnoid space receives a modicum of fluid directly from the brain tissue itself.

In order to understand this latter possibility it is necessary to consider the relationship of the pia to the surface of the brain and to the penetrating vessels. In the mammalian embryo, as Weed has shown, the pia arises as a condensation of mesenchymal tissue in contact with the neural tube. As the adult structure of the leptomeninges shapes itself, this pial condensation becomes reduced gradually to a single layer of mesothelial cells covering the neural tissue. This layer is continuous, except that it is penetrated in a multitude of places by the perforating arterioles and venules from the intraleptomeningeal vessels. As each vessel perforates the pia to enter the brain substance it carries with it, besides its vascular coats proper, a delicate sheath of mesothelial cells derived from the arachnoid. At the same time the pial mesothelium, at the edges of the tiny funnel in the brain substance which accommodates the vessel, is carried inward as a delicate cuff-like lining. Thus, there is formed about each vessel a sheath-like space, the

so-called perivascular space which is lined near the surface of the brain by mesothelial prolongations from the subarachnoid space. These mesothelial investments accompany the vessel for several millimeters when they dwindle and disappear. The perivascular space, however, accompanies the vessels along all its ramifications and eventually communicates directly with the entire system of pericellular spaces and tissue spaces within the brain. These relationships are represented diagrammatically in the accompanying illustration (Fig. 508, from L. H. Weed). His (1865) was the first to establish fully by means of puncture injections the presence of this system

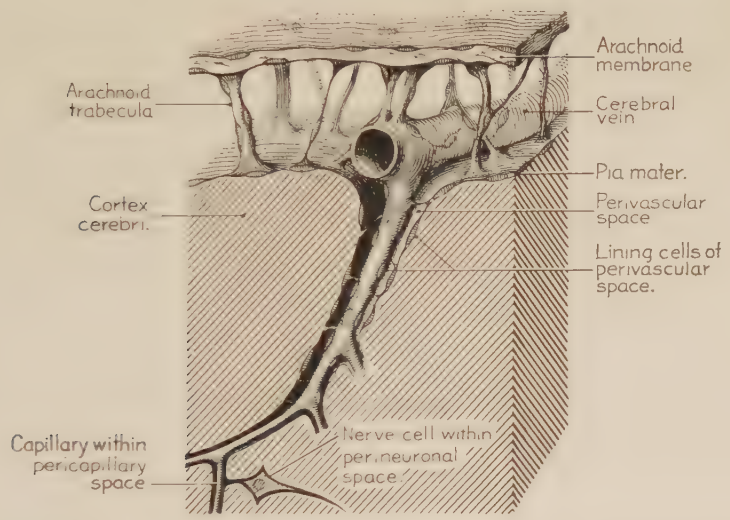


FIG. 508.—Schematic diagram of leptomeninges and nervous tissue, to show relation of subarachnoid space, perivascular channels and nerve cells. The leptomeningeal lining cells are reflected inward for various distances to form lining cells of the perivascular space. (Weed, 1923.)

of richly inter-communicating spaces in the nerve tissue and around the blood vessels. He was able to demonstrate that each nerve cell is surrounded by a pericellular or perineuronal space which communicates with the perivascular spaces. Both in the cord and in the brain he observed that the injection fluid spread peripherally through the tissue spaces and perivascular channels towards the pia. The conception of such an intercommunicating network of spaces has been amply substantiated by the observations of Spina (1899), Bruno (1899), Jacob (1900), Lewandowsky (1900), Milian (1904) and Poirier and Charpy (1907). Spina (1899) observed the exudation of clear droplets of fluid from the surface of the brain following marked rises in blood pressure produced by injecting extracts of the adrenal glands. Lewandowsky (1900) interpreted Spina's observation as evidence that the

cerebrospinal fluid is really the lymph of the central nervous tissue and that it accumulates in the subarachnoid space as a product of the perivascular spaces.

Mott (1910) observed that in the brains of monkeys, in which a cerebral anemia has been produced by ligation of the carotid or vertebral arteries, the perivascular and perineuronal spaces were tremendously dilated. His observation gave further convincing proof of the existence of potential communications between the subarachnoid space and the brain tissue. He hypothesized from this the passage of cerebrospinal fluid from the subarachnoid space via the perivascular channels into the cerebral capillaries. Thus he contended that these pathways represent normally the mode of escape of the cerebrospinal fluid into the blood vessels.

Weed (1914,1923) has investigated the question of the nature of these pathways using the injection of a true solution under physiological conditions as his method of approach. On injecting the Prussian blue reagents by replacement into the subarachnoid space of the adult cat or dog within the range of normal pressures, the injected fluid spreads rapidly throughout the leptomeningeal spaces, but fails to penetrate the perivascular spaces. Weed has found that under these conditions the absorption of cerebrospinal fluid in the cranium occurs almost exclusively via the arachnoid villi into the great dural sinuses.

If, on the other hand, the pressure conditions within the cranium were altered experimentally, Weed could produce a retrograde injection of cerebrospinal fluid into the perivascular spaces. So, for instance, if the animal was exsanguinated after replacement of the cerebrospinal fluid by the true solution, the Prussian blue reagents seeped rapidly into the entire perivascular and perineuronal system. Also after injecting a strongly hypertonic solution of sodium chloride into the circulation of an animal, the cerebrospinal fluid, as evidenced by the presence of Prussian blue, became aspirated into the perivascular channels.

Weed explains his observation upon the very plausible hypothesis that so long as normal relationships exist between arterial, venous and cerebrospinal fluid pressures within the closed cranium, the stream of tissue fluid is from the neuronal and perivascular spaces towards the subarachnoid space. As soon, however, as the arterial pressure becomes lower than that of the cerebrospinal fluid, as for instance by exsanguination, a reversal of the normal flow is accomplished. A similar reversal may be attained by increasing the osmotic pressure of the blood by the injection of hypertonic salt solution which results in the attraction of cerebrospinal fluid into the circulation by every available pathway. Under normal conditions, then, we must conceive of a flow of fluid, probably conveying waste products from the neurons, towards the subarachnoid space via the perivascular channels. This is in effect what Lewandowsky postulated, but on insuffi-

cient grounds. Moreover, he, as well as the majority of writers on this subject, homologize the spaces within the nervous tissue and their contained fluid with lymph spaces and lymph, a correspondence which as Weed emphasizes is quite inadmissible in the light of our modern conception of the nature of lymphatic vessels and lymph. The cerebrospinal fluid is a special body liquid differing entirely in its low protein and cellular content from true lymph.

VII. THE CYTOLOGY OF THE LEPTOMENINGES

The subarachnoid space, as we have traced it, is composed of a system of partially interrupted spaces, completely lined by mesothelial cells. These spaces receive the cerebrospinal fluid from its dual sources, the ventricles and the perivascular spaces. It has been demonstrated that the mesothelial cells lining the subarachnoid cavities are efficient fluid retainers. All of the experiments, beginning with those of Key and Retzius, and Quincke, and concluding with those of Weed, demonstrate that the cellular lining of the leptomeningeal spaces, except for certain specialized parts, which will be described in reference to the absorption of the cerebrospinal fluid, are under normal functional conditions relatively impermeable to the passage of true solutions or particulate suspensions by diffusion or filtration. This capacity of the pia arachnoid to retain the cerebrospinal fluid is established, according to Weed, from the time the fluid escapes in the embryo into the loose mesenchyme between the two blastemal condensations which subsequently give rise to the leptomeninges.

The cerebrospinal fluid, contained normally within the meshes of the pia arachnoid, is a clear fluid, almost devoid of cells. However, on irritation of the meninges, by the introduction of bacteria, toxins or particulate matter into the cerebrospinal pathway, cells are stimulated to migrate in large numbers into the subarachnoid space. It has been generally conceded in the past that these cells belong to the categories of polymorphonuclear cells, mononuclear cells and lymphocytes which are derived either from the blood stream directly, or from the perivascular tissue or from the stroma of the pia arachnoid (Quincke, 18–2; Mertzbacher, 1905; Widal, Sicard and Ravaut, 1901; Fuchs and Rosenthal, 1904; Alzheimer, 1907). However, in 1917, Weed reported the observation that particles of carbon or cinnabar introduced in the subarachnoid space were phagocyted by the mesothelial cells lining the pia arachnoid. Essick (1920) then undertook to study the rôle of the mesothelial cell in the production of phagocytic cells in the cerebrospinal fluid of the cat. He injected from 1 to 6 c.c. of sterile, partially laked red blood corpuscles into the subarachnoid space by replacement of fluid. There ensued in the following six hours a sterile meningitis accompanied by the outpouring of polymorphonuclear

and transitional cells. In the next forty-eight hours the polymorphonuclear forms almost disappeared, while the mononuclear elements increased in number. Both types of cell were actively phagocytic from the start, taking up large numbers of fragments of the foreign red blood corpuscles into their cytoplasm.

Essick made skillful dissections in the form of spread preparations of the pia arachnoid in which he could study the mesothelial cells. By this method he was able to ascertain that the mesothelial cell, while still in situ on the arachnoid trabecula, engulfed particles of red blood cells and that it became changed in form, eventually rounding up, and finally dropping off, as a free phagocytic mononuclear cell into the cerebrospinal fluid. Among the still attached mesothelial cells abundant mitotic figures gave evidence of rapid replacement of these elements. He concluded his report by stating that particulate matter within the subarachnoid cavity causes the lining mesothelial cells to round up and bud off from their attachments and that as free moving ameboid elements these cells fulfill in every way the criteria of the class of macrophages and as such are concerned in the removal of débris. It may be mentioned that Ayer (1920), working contemporaneously with Essick, reported a similar change in the morphology of the mesothelial cells in cases of acute experimental meningitis due to bacterial toxins. The accompanying illustrations (Fig. 509, from C. R. Essick) depict the normal mesothelial cells covering the arachnoid trabeculae, as well as different stages in their response to irritation.

Woollard (1924) next investigated this question using trypan blue, a dye which Goldmann (1913) had shown stained the leptomeninges when injected in the subarachnoid space. Woollard employed one-tenth of one per cent of the dye made up with Ringer's solution, giving repeated injections of 6 to 8 c.c. by replacement through an occipito-atlantoid puncture. No free cells were obtained from the cerebrospinal fluid during the course of the experiment, but at autopsy the leptomeninges were found deeply stained, besides a fainter coloration of the brain and cord itself. On making spreads of the arachnoid, similar to Essick's, Woollard ascertained that the dye had penetrated the leptomeninges and had been taken up in the form of cytoplasmic granules in two types of cells. In the mesothelial cells the dye was as a rule sparse, staining the cytoplasm near the poles of the nucleus, but he found some mesothelial cells which showed evidence of rounding up and assuming the appearance of mononuclear phagocytes resembling clasmatocytes (macrophages). Besides this, he found more deeply stained cells possessing the appearance of characteristic clasmatocytes (macrophages) in the trabeculae and membranes, which he believed were distinct from and in no way derived from the mesothelial cells. The typical fibroblast he found concerned in taking up the dye in only a minor way. He then tried the combined experiment of injecting trypan blue and laked

red blood cells. He corroborated the result of Essick, that the mesothelial cells are stimulated to produce mononuclear phagocytes, but he became convinced of the simultaneous production of similar phagocytes (clasmatocytes) from cells in the stroma of the meninges and in the perivascular spaces.

FIG. 509.—A, normal flattened appearance of cells covering the arachnoidal trabeculae. B, three stages in transformation of arachnoidal cells into macrophages occurring after injection of erythrocytes: (a) Primary increase in protoplasm about the nucleus. (b) beginning formation of a meshwork. (c) swelling up preparatory to budding off. C, initial swelling of protoplasm as shown in preparations stained with aqueous toluidine blue. D, phagocytosis of fragmented erythrocytes and blood pigment by cells covering arachnoidal trabeculae. E, division of arachnoidal cell on trabecula. F, G and H, phagocytic macrophages from subarachnoid space. I, macrophage from subarachnoid space. J, ameboid macrophage from subdural space. (Essick, 1920.)

Finally Kubie and Schultz (1925) undertook an analysis of the cells occurring under normal and experimental conditions in the cerebrospinal fluid of the cat. They, too, availed themselves of trypan blue which they made up, instead of as Woollard had done in Ringer's solution, in distilled water, because they considered the dye less toxic to the central nervous system when administered in this form. Doses of 1 to 3 c.c. of a quarter to a

one per cent solution were given by replacement. They made a survey of the cells present in the fluid during the course of each experiment using the supravital technique of Sabin, Cunningham and Doan (1923, 1925) for the differentiation of living blood and connective tissue cells.

The first response to the injected dye stuff Kubie and Schultz found was an outpouring of polymorphonuclear neutrophile cells which gradually disappeared, concomitantly with the appearance of mononuclear cells. By the supravital technique, with neutral red and Janus green, they were able to differentiate in drops of the cerebrospinal fluid, or in scrapings of the arachnoid, the following cells: polymorphonuclear neutrophiles, polymorphonuclear eosinophiles, lymphocytes (small, intermediate and large), mononuclear phagocytes and arachnoid lining cells. In the films of cerebrospinal fluid which they studied, the polymorphonuclear neutrophiles were less actively motile and degenerated more quickly than in similar preparations of normal blood and, moreover, they did not stain vitally with trypan blue. Eosinophile leucocytes occurred rarely in the cerebrospinal fluid. They were non-motile and did not undergo degeneration as the neutrophilic cells did. The lymphocytes of the cerebrospinal fluid, they found to be derived from the perivascular spaces of the brain and cord in which, after irritation, they accumulated in great numbers. Few of them found their way spontaneously into the cerebrospinal fluid, but large numbers of them became dislodged and appeared in the subarachnoid space when part of the cerebrospinal fluid was drained by occipito-atlantoid puncture. Following Sabin's observations on blood, they described three kinds of lymphocytes in the cerebrospinal fluid, none of which stained vitally with trypan blue. The intermediate lymphocyte was the only motile lymphocytic form in the cerebrospinal fluid; the large lymphocyte was rarely encountered. Plasma cells, which Kubie and Schultz traced from the intermediate lymphocyte, were also present, after irritation, in the cerebrospinal fluid and the perivascular sheaths.

The mononuclear phagocytes of the cerebrospinal fluid and meninges became heavily stained with trypan blue. By the supravital technique of Sabin, Kubie and Schultz were able to distinguish two types of mononuclear phagocytes, clasmatocytes and monocytes, in the cerebrospinal fluid, in keeping with such a distinction made in blood and connective tissue by Sabin, Cunningham and Doan.

By the supravital technique, by the spread dissections of Essick, by ordinary sections and by tissue cultures they attempted to determine the origin of these mononuclear phagocytic elements (macrophages of other writers). They became convinced from their studies that the monocyte encountered in the cerebrospinal fluid must have arisen primarily by the multiplication of inactive monocytic forms which seemed normally to inhabit the arachnoid. Similarly, they decided that the clasmatocytes arose

by the multiplication of immature forms which inhabit the perivascular sheaths.

The arachnoid lining cell they found did not stain supravitally with Janus green and it took up no neutral red unless subjected to irritation. This failure to react towards Janus green and neutral red is unlike the behavior of typical monocytes or clasmatocytes or the young forms from which they are derived. Moreover, Kubie and Shultz found the characteristics of the arachnoid mesothelial cell to be in many ways similar to those of the peritoneal lining cells as described by Cunningham (1922, 1924). The latter investigator, in a recent series of excellent experimental studies on the peritoneal cavity, has concluded that the peritoneal mesothelial cell, contrary to the generally accepted view, is not actively phagocytic, nor is it the source of the free macrophages of serous exudates. After repeated injections of trypan blue, Kubie and Schultz found that the living arachnoid cell acquired a fine stippling of blue, but they were quite unable to accept the view of Essick and Woollard that transitions occurred between the mesothelial cell in this condition and the active, heavily laden phagocyte. They state that what transformation the living arachnoid cell seemed to undergo with irritation was rather towards the fibroblast than towards the macrophage.

It is generally accepted that the lymphocyte is the only cell constituent of normal cerebrospinal fluid. However, both Essick, and Kubie and Schultz have found an occasional phagocytic cell in the normal fluid of the cat, which the latter by the supravital technique identify as young monocytes.

In trying to reconcile the conflicting results of Essick, Woollard, and Kubie and Schultz in regard to the rôle of the mesothelial cell in irritation of the meninges, it is scarcely necessary to point out the difficulty which one encounters in the interpretation of whether cells are in the act of rounding up and dropping off the surface of a mesothelial membrane or are in the process of passage through the surface; witness the conflicting interpretations elsewhere concerning the derivation of phagocytic cells from endothelial walls or serous surfaces. Quite aside from this, however, one is struck at once by a dissimilarity in the experimental procedures of these investigators. Essick dealt with particulate matter in the form of fragmented erythrocytes, Woollard, and Kubie and Schultz with a dye-stuff which in distilled water forms an ultramicroscopic sol. Woollard, however, made his dye up in Ringer's solution, a procedure which as any one knows who has handled this dye results in partially salting the dye out or at least in converting it into a less dispersed form than in distilled water. Hence Essick was dealing with a relatively non-diffusible substance, Woollard with a somewhat diffusible one, and Kubie and Schultz with a relatively readily diffusible one. Hence the radius of action of the three experimental substances within the meninges may have been different. The suspension of

red blood cells on this assumption will have been most localized in its action, the trypan blue as prepared by Woollard less so, and that of Kubie and Schultz most widely diffusible. Moreover, it cannot be denied that trypan blue is slightly toxic, its toxicity increasing naturally with the state of its dispersion. Furthermore, although the mesothelial cells constitute undoubtedly a confining membrane in terms of fluid pathways from the subarachnoid space, yet it seems quite improbable that true solutions as well as molecular sols do not slowly penetrate them. Undeniably, trypan blue, as shown by Woollard's and Kubie's experiments, penetrates the mesothelium and stimulates the subadjacent tissue, whether as the result of one or both of the above-mentioned possibilities one cannot say. Hence we believe it is quite probable that in Essick's experiments the stimulus is largely on the mesothelium, in Kubie and Schultz's predominantly on the outlying clasmatocytes and monocytes, while in Woollard's experiment the effect is intermediate. It is much to be hoped that this problem will be reinvestigated, full consideration being given to the differences in technique of the previous investigators. Kubie and Schultz are to be complimented on their application of the nomenclature current in modern studies on blood and connective tissue, to the cytology of the meninges and perivascular spaces whenever this is possible. The fact that the meningeal envelopes and perivascular sheaths are but a derivative of the general mesenchyme and hence identical with connective tissue in many of their fundamental cellular reactions is lost sight of by many neurohistologists.

The observations of Weed, Essick, Ayer and Woollard give us an illustration of how the morphology of the leptomeningeal cells depends not only upon their situation, but also upon their physiological state. Further examples of this will become apparent in discussing the modification which the mesothelium undergoes in the formation of the arachnoid villi and the cell clusters in the angles of the spinal nerve roots, characteristically modified mesothelial areas which subserve the drainage of the cerebrospinal fluid.

Moreover, the mesothelium of the arachnoid is subject to a morphological change with advancing age. Cushing and Weed (1915), Essick (1920) and Weed (1920) have described and commented upon characteristic cellular hyperplasias of the arachnoid mesothelium in adult man and animals. By a multiplication of the cells of the normally single-layered mesothelium, small clusters of cells are produced in places throughout the cranial and spinal arachnoid which in many instances lead to a fusion of the arachnoid with the dura and often to an invasion of the dura by columns of cells. In more advanced age these areas may undergo calcareous or osseous transformation or give rise by more extensive proliferation to typical endotheliomata. That these clusters of mesothelial cells are physiologically as well as morphologically changed is shown by the fact that they are partially penetrated by replaced ferrocyanide solution, perhaps the only reason for

their failure to be completely permeable being that they do not develop in contiguity to absorbing venous channels.

VIII. THE ARACHNOID VILLI

The arachnoid is separated from the inner surface of the dura by the narrow subdural space except in certain definite areas in which a fusion of the two membranes occurs. These places of union arise by invasion of the pachymeninx by the cells of the arachnoid, principally along the courses of the dural sinuses. In these areas projections of the arachnoid occur into the walls of the sinuses. It is in these regions, as is now generally accepted, that the major absorption of cerebrospinal fluid takes place.

Macroscopic projections into the dural sinuses were first discovered by Pacchioni in 1741. He described in man peculiar warty excrescences protruding into the lumen of the longitudinal sinus which he interpreted as a kind of glandular structure. These, the "glandulae Pacchioni" or Pacchionian corpuscles of later writers, Burdach (1819 to 1822), Cloquet (1828) and Cruveilhier (1845) were unable to find in embryos and young individuals, and Todd (1847) did not succeed in finding them at all in animals. Luschka (1855), by the aid of the microscope, distinguished their origin from the arachnoid and designated them "arachnoidal villi." This conception was corroborated by subsequent investigators, notably by Key and Retzius (1875). In spite of this, their absence in young individuals and in animals was universally accepted and much discussion arose as to whether they were normal structures in man or not.

It was not until quite recently and in connection with the whole question of the absorption of the cerebrospinal fluid that the real nature of the close association of the arachnoid with the dural sinuses has become better understood. Our knowledge of the escape of the cerebrospinal fluid from the subarachnoid space begins with the observation of Key and Retzius that in cadavers in which they injected a gelatin mass, containing Berlin blue, under considerable pressure into the subarachnoid space they observed the spread of the gelatin throughout the continuous intraleptomeningeal spaces. Furthermore, they were able to trace the injection mass into the cores of the Pacchionian bodies and often through these structures actually into the lumen of the dural sinuses. In spite of the fact that Pacchionian granulations do not exist in infancy or in animals, their observations were generally accepted as establishing the pathway of escape of cerebrospinal fluid from the subarachnoid space. They also discovered, in addition to what they considered to be the major pathway of absorption into the dural sinuses, a minor escape of fluid into lymphatics by way of the perineural spaces of the cranial nerves.

Further investigation of this question was deferred until Reiner and Schnitzler (1894, 1895) showed that potassium ferrocyanide could be recovered from the jugular vein in from thirty to forty seconds after injection into the subarachnoid space of living animals. They succeeded likewise in identifying injected olive oil in a similar way. Next Hill (1896) was able to follow the passage of methylene blue from the subdural space into the venous sinuses and to recover it in the urine and stomach. Similarly Ziegler (1896) got a positive test for potassium ferrocyanide in blood, taken from the posterior facial vein, ten seconds after injection, and only after thirty minutes in the cervical lymphatics. Lewandowsky (1900) also identified sodium ferrocyanide in the urine in thirty minutes after injection. Likewise Spina (1900 to 1901), after introducing a solution of fuchsin into the subarachnoid spaces of animals recently dead, demonstrated a major drainage into the dural sinuses and a minor lymphatic drainage. Cushing (1902) also traced the

passage of mercury globules, as well as non-absorbable gases, into the dural sinuses and postulated the presence of a valve-like mechanism governing their transmission. These observations all involved the venous channels in the dura in some incompletely understood way in the absorption of cerebrospinal fluid.

Mott, however, in 1910, from his observations of the brains of monkeys in which the perivascular and perineuronal spaces of the brain tissue were widely distended as a result of an experimentally produced cerebral anemia, hypothesized the absorption normally of cerebrospinal fluid from the subarachnoid space into the cerebral capillaries via these channels. As has already been discussed, the observations of Weed failed to substantiate the view of such an absorption under normal conditions.

Cathelin (1912), on the other hand, became the exponent of absorption from the subarachnoid space solely by way of lymphatics, and Goldmann (1913) gave much support to such a view from his studies on the absorption of trypan blue, although he could not deny the evidence in favor of the major absorption via the dural veins.

Finally Dandy and Blackfan (1913, 1914) arrived at the conclusion, based upon the results of injection of phenolsulphonephthalein, that the drainage of cerebrospinal fluid takes place by a diffuse process of absorption from the entire arachnoid space. They found that when the spinal subarachnoid space was completely isolated from the cranial, a quantitative absorption proportionately as great as from the entire subarachnoid space occurred. India ink, however, did not undergo absorption. Their view is not in accord with the prevailing conception of the absorption of cerebrospinal fluid and has several obvious objections to be raised against it. The work of Weed more particularly, and of others, has shown that the limiting membranes of the subarachnoid spaces are permeable to too slight an extent, if at all, under normal conditions, to allow absorption to occur. Moreover, the arachnoid membrane is avascular in the sense of possessing a capillary bed into which drainage might occur, and furthermore Weed has not observed the passage of the Prussian blue under normal conditions directly into the veins or arteries traversing the leptomeninges. In reference to absorption from the isolated spinal cord, escape of spinal fluid along the nerve roots, as will be described presently, seems a more likely explanation than the one offered by Dandy and Blackfan.

The foregoing brief outline portrays the uncertainty concerning the pathway of absorption of cerebrospinal fluid and the complete ignorance of the cellular structures involved in its drainage at the time when Weed (1914) started to investigate the problem. Using balanced, isotonic solutions of potassium ferrocyanide and iron ammonium citrate, he replaced the cerebrospinal fluid, taking every precaution to maintain normal cranial pressures during the course of the experiments. By his adequately controlled experiments on cats, dogs and monkeys, he was able to demonstrate that under normal conditions the major drainage of the cerebrospinal fluid is into the dural sinuses of the skull while there is an accessory drainage along some of the cranial nerves into the lymphatic system.

With the Prussian blue reagents at hand he had an opportunity to trace the actual course of the fluid through the tissue from the subarachnoid space into the dural veins. This necessitated an anatomical study of the arachnoid in the region of these sinuses, the result of which has given us a more complete understanding of the mechanism of absorption. In all the mammals which he studied, including the meninges of human infants, he

found a curious penetration of the dura in the neighborhood of the venous sinuses by villous evaginations of the arachnoid to which he gave the designation "arachnoid villi." These structures were mostly hollow, finger-like evaginations, but ofttimes consisted of sleeve-like investments about the cerebral veins at their points of entry into the sinuses, while in the dog they were more like villous strands which had become flattened out against the wall of the sinus. All of these modifications, however, were alike in that they were hollow and contained a core of loose-meshed, arachnoidal filaments, myxomatous in character, the interstices of which were in open communication with the subarachnoid space. Moreover, these villi were in intimate contact with the endothelial wall of the sinus, either flattened out against it, or actually protruding into the lumen of the vein. Arachnoidal mesothelial cells entered the sponge-like cores of the villi, supplying its walls with a lining of flattened cells. The apex of the villus which was in contact with the endothelium of the vein was formed by a cap or little cluster of arachnoid cells. The dura was completely perforated by these villi and did not contribute to the composition of the actual villus, nor could the subdural space be traced any farther than to the stalk or root of the villus, at which point it seemed to disappear. By such an arrangement, then, the arachnoid and the subarachnoid space were brought into the immediate proximity of the endothelium lining the dural sinuses (Fig. 510, from L. H. Weed).

These structures could be brought into close harmony with the previously known macroscopic villous projections or Pacchionian bodies, for Weed could trace changes whereby the microscopic arachnoid villi, as he had found them in both animals and human infants, underwent hypertrophy with advancing age of the animal, which in man resulted finally in the production of typical Pacchionian granulations.

Employing the Prussian blue reagents by substitution for cerebrospinal fluid under exact pressure control, Weed (1914, 1920) traced the major escape of the Prussian blue into the dural sinuses through the arachnoid villi. Microscopic examination of these structures, during the passage of the foreign solution, gave evidence of the direct passage of the salts from the hollow cores of the villi through the arachnoidal mesothelial cells, and the delicate endothelium of the vein, into the circulating blood. Although there was some indication that passage of the foreign solution occurred between the cells, as well as through them, the presence of stomata or stigmata could be ruled out. Presumably the passage of gelatin through actual stomata in the Pacchionian bodies, as described by Key and Retzius, can be attributed to the formation of artefacts by the abnormally high pressures which they exerted.

From a wide experience with the physiological factors involved in the formation and drainage of the cerebrospinal fluid, Weed (1923) con-

cluded that in the normal process of absorption of cerebrospinal fluid into the great veins, filtration may be the factor of greatest importance, by the passage of the fluid from a point of higher pressure (subarachnoid space) to a point of lower pressure (dural venous sinus) through the cellular membranes of the arachnoid villi. However, Weed (1923) was able to show that after the intravenous injection of strongly hypertonic saline solutions the osmotic pressure of the blood becomes extreme, and that osmosis and diffusion become operable under such abnormal conditions in the absorption of the foreign solution from the subarachnoid space. It

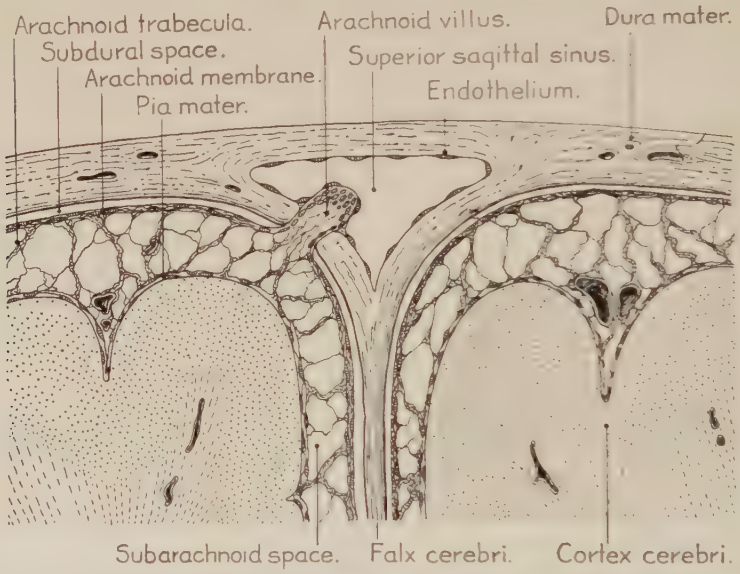


FIG. 510.—Schematic diagram of coronal section of meninges and cerebral cortex, to show relation of arachnoid villus to dural venous sinus. The potential subdural space is necessarily shown of greater size than is normal; the subarachnoid space over the convolutions is also increased in width to illustrate the character of the subarachnoid mesh. (Weed, 1923.)

may be presumed, however, that under normal conditions the latter factors play a negligible rôle in the absorption of the cerebrospinal fluid.

In the same year with the publication of Weed's first observations upon the drainage of the cerebrospinal fluid, Frazier and Peet (1914) reported the results of injections of a number of dyes into the subarachnoid space. They concluded that the mode of escape of these substances was largely into the venous system, while the lymphatic drainage played only a minor rôle.

Several years later, Dixon and Halliburton (1916) reported the results of a long series of observations upon the absorption of substances from the

subarachnoid space. True solutions they found were absorbed rapidly, colloidal solutions more slowly, while particulate matter remained localized within the leptomeninges. They concluded that the major escape of fluid occurs into the blood-vascular system of the cranium, and Halliburton (1916) accepts Weed's view by stating that the fluid probably reaches the venous sinuses by way of the microscopic arachnoid villi. On the other hand, they could not find evidence of lymphatic absorption by testing lymph from the thoracic duct.

IX. THE CRANIAL AND SPINAL NERVE ROOTS

Opinion differs greatly as to the degree of accessory drainage of cerebrospinal fluid into the lymphatic system, some practically denying this mode of escape, others assigning it a major rôle. All are agreed, with the exception of Mott and Dandy and Blackfan whose views concerning absorption need not be rediscussed, that lymphatic drainage takes place along the cranial and spinal nerve roots. Hence, any real settlement of the problem concerns a study of the manner of passage of substances from the subarachnoid space out along these nerve trunks. With the exception of certain of the cranial nerves, no accurate studies of the morphology of the nerve roots in relation to the passage of substances along them from the subarachnoid seem to have been undertaken. An advance in this direction has been made for the first time only recently by Elman (1923) and by Wislocki and Kubie in unpublished observations as a result of the combined experimental and morphological study of the spinal nerve roots. Many of the previous observations were carried out by naked eye inspection of the spinal roots or simply by identification of some substance in the thoracic duct lymph or lymph nodes.

For many years it has been held that extensions of the arachnoid membrane extend out along the cranial and spinal nerves and for a long while it was believed that there were direct anatomical communications between these cuff-like extensions of the subarachnoid space and the so-called perineural spaces around the peripheral nerve trunks. Key and Retzius (1875) believed this from the spread of injected material from the subarachnoid space into the perineural spaces. Their view can be assailed, however, on the basis of rupture of the delicate membranes under the high pressure of injection, producing artefacts rather than indicating true anatomical pathways. More recent writers have abandoned the idea of direct anatomical connections, particularly Weed (1914) who has shown that suspended material such as carbon granules, injected within the range of normal pressures, remains confined to the subarachnoid space. Other experiments of a similar kind, for instance those of Quincke (1872) with cinnabar, Sicard and Cestan (1904) with carbon particles, Goldmann (1913) using trypan blue and Flexner and Amoss (1914) injecting carmine granules, add little real information, because the materials which they used were left in the meninges long enough to produce irritation and to undergo subsequent transport by phagocytes. The conception of anatomical communications has given way in recent years to the belief in a functional pathway along the spinal nerves, involving, in an insufficiently demarcated way, however, the passage of

fluid through the closed arachnoid into the perineural spaces and ultimately into lymphatics surrounding these spaces. Cathelin (1912) describes the cerebrospinal fluid as passing out along the spinal nerves to be absorbed by the lymphatics, and he says of this rather hypothetical route that the peripheral nerves are bathed to their very termination by the same fluid that covers the central nervous system. Weed (1914) gave no particular study to the mode of absorption of fluid from the spinal subarachnoid space, although his results are stated to be in accord with the pathway suggested by Sicard and Cestan (1904), which involves the utilization of perineural spaces with ultimate absorption into the lymph stream. He says that such a mechanism of lymphatic drainage may be demonstrated in the long-continued low pressure injections with the ferrocyanide method in an isolated portion of the spinal cord. It may be stated, however, that Weed believes that under normal conditions the amount of cerebrospinal fluid absorbed from the spinal subarachnoid space is a minimal quantity.

Elman (1923) undertook to study the morphology of the meninges at the points of penetration of the nerve roots in conjunction with the use of replacement injections of cerebrospinal fluid with carbon and the Prussian blue reagents. From his observations with carbon, he reaffirms the opinion of Weed, that the arachnoid constitutes a limiting membrane towards particulate matter and that no anatomical continuity exists between the spinal subarachnoid space and the spaces surrounding the nerves. By the ferrocyanide method, however, he was able to trace the foreign fluid through the arachnoid into the tissue surrounding the nerve roots.

On microscopic examination of his material he discovered that the arachnoid does not accompany the roots for any distance as a cul-de-sac, but that it is reflected at a sharp angle instead, as a complete investment for both posterior and anterior roots and carried centrally along them to fuse with the pial covering of the spinal cord. Furthermore, he found that, in the region where the arachnoid becomes applied to the nerve roots, the arachnoidal mesothelium has proliferated seemingly to form clusters of cells. It is these clusters of cells at the angles of reflection of the arachnoid that the foreign solution penetrates. This is in keeping with the penetration of the similarly modified arachnoid villi in the cranium, as well as of arachnoidal clusters of cells, described by Essick (1920) and Weed (1920), which occur not infrequently in the meninges of man and animals. The latter, as shown by Weed, exhibit under high pressures a partial penetration of the ferrocyanide solution.

In the spinal cord the similar clusters of cells, however, appear to be concerned in the transmission of fluid from the subarachnoid space into the tissue around the nerve roots. Elman could not trace the foreign solution into the perineural spaces, but could follow it quite definitely into vessels around the nerve roots which in some instances he could identify as veins. That some of these vessels might be lymphatics he had no way of denying.

Recently, in studying the spinal cords of cats in which the blood vessels had been injected with India ink or carmine gelatin and the specimens cleared or cut, Wislocki and Kubie have studied the vessels in the region of

the nerve roots. There appears to be no doubt from study of these specimens that, as the nerve roots penetrate the arachnoid and dura successively, the roots are surrounded by a plexus of dural veins, amounting to sinuses. Each segmental artery as it accompanies the spinal nerve between the vertebrae can be seen to give off several arterial twigs to the dura. These can be traced into a capillary bed within the dura between adjacent segments. From this bed the blood drains into slender veins, two of which accompany each artery. These veins approach the region of the nerve exits from the dura and as they reach the region of the dura enclosing the penetrating nerve roots, they enlarge slightly and suddenly enter a series of communicating sinuses in the dura encircling the nerve roots. One or several short, wide veins drain from the circle of sinuses into the plexus of peridural veins in the vertebral canal.

From these observations it seems likely that the normal absorption of cerebrospinal fluid along the spinal nerves is similar to the manner of drainage in the skull. It must be admitted, however, that in the long-continued low pressure injections in the isolated spinal cord as performed by Weed, the ferrocyanide solution eventually reaches the perineural spaces beyond the dura and undergoes absorption by ways of lymphatics.

Much of the evidence of lymphatic absorption via the spinal nerves by previous writers is open to criticism. As Elman has pointed out, most of them used uncontrolled pressures. Furthermore, the recovery of a foreign solution in the thoracic duct lymph several hours after spinal injection can be interpreted otherwise than indicating drainage from the meninges into the lymph. It might very well be that phenolsulphonephthalein, as an example, had left the meninges by venous drainage entirely, but by general dispersion through the body by conveyance in the blood stream could subsequently be identified in the thoracic duct lymph. Finally, as having a bearing on this question, Essick and Kubie and Schultz may be quoted to the effect that it is extremely difficult to perform a lumbar or occipito-atlantoid puncture of the meninges without getting seepage of the ferrocyanide solution into the tissues surrounding the dural puncture with subsequent coloration of the regional lymphatics and lymph nodes.

In this connection it can be only briefly stated that on the authority of the best observers lymphatics do not occur within the meninges. In the earlier literature on the meninges the presence of lymphatics was accepted, as the result of injections of anastomosing channels in the pia arachnoid and dura, by Arnold (1838), His (1865) and Böhm (1869). In the light of our modern conception of the lymphatic system their injections must all be regarded as the filling of tissue spaces under immoderate pressures. Sabin (1912) in studying the growth of the lymphatic system in embryos has not observed the penetration of lymph vessels into the dura, nor has Weed (1914) found any evidence of their presence in the meninges.

Our discussion applies so far only to the spinal nerves. Amongst the cranial nerves, two at least, the olfactory and optic nerves, show a constant and characteristic drainage along their peripheral courses. Weed (1914) gives an excellent account of the passage of the ferrocyanide solution along these nerves. Following an injection of the solution into the subarachnoid space, precipitated Prussian blue can be followed into the perineural spaces surrounding the olfactory nerves and traced through the cribriform plate into the walls of the nasal cavity, where it spreads into the connective tissue of the nasal mucous membrane. Thence the solution undergoes absorption into lymphatic channels draining into cervical lymph nodes. Using granular material it is impossible under normal pressure to secure injections of the olfactory nerves beyond the cribriform plate.

About the optic nerves, injected material spreads along the infravaginal (subarachnoid) space to communicate with the posterior wall of the eyeball. Here the foreign solution can be identified in the epichorioidal space and in the episcleral space of Schwalbe. Finally, the foreign material can be traced around the bulb towards the filtration angle at the corneoscleral junction. For the correspondence between the processes of drainage of cerebrospinal fluid and the ocular humors the reader is referred to the interesting papers of Wegefarth and Weed (1914).

X. BIBLIOGRAPHY

Alzheimer, A. 1907. Einige Methoden zur Fixierung der zelligen Elemente der Cerebrospinalflüssigkeit. *Zentralblatt f. Nervenheilk. u. Psych.*, **30**, 449.

Arnold, F. 1838. *Bemerkungen über den Bau des Hirn- und Rückenmarkes.* Zürich.

Ayer, J. B. 1920. A pathological study of experimental meningitis from subarachnoid inoculations. *Monographs of the Rockefeller Institute*, **12**, 26.

Becht, F. C. 1920. Studies on cerebrospinal fluid. *Amer. Jour. Physiol.*, **51**, 1.

Blake, J. A. 1900. The roof and lateral recesses of the fourth ventricle considered morphologically and embryologically. *J. Comp. Neurol.*, **10**, 79.

Böhm, R. 1869. Experimentelle Studien über die Dura Mater des Menschen und der Saügethiere. *Virchow's Archiv f. path. Anat.*, **47**, 218.

Bonne, C. 1899. Note sur le mode d'oblitération partielle du canal épendymaire embryonnaire. *Rev. neurol.*, **17**, 114.

Bruno, J. 1899. Ueber die Injection von Giften ins Gehirn. *Deutsch. Med. Wochensch.*, **25**, 369.

Burdach, 1819–22. *Vom Baue und Leben des Gehirns.* Leipzig.

Cappelletti, L. 1900. L'écoulement de liquide cérébrospinal par la fistule céphalorachidienne en conditions normales et sous l'influence de quelques medicaments. *Arch. Ital. de Biol.*, **35**, 463.

Carlson, A. J., and Martin, L. M. 1911. Contributions to the physiology of lymph. XVII. The supposed presence of the secretion of the hypophysis in the cerebrospinal fluid. *Am. J. Physiol.*, **29**, 64.

Cathelin, F. 1912. *La circulation du liquide céphalo-rachidien.* Paris: J. B. Bailliére et fils.

Claisse, P., and Levy, C. 1897. Etude histologique d'un cas d'hydrocephalie interne. *Bull. de la Soc. anat.*, 265.

Cloquet. 1828. *Traité d'anatomie descriptive.* T. 2, Paris.

Cruveilhier. 1845. *Traité d'anatomie descriptive.* T. 4, Paris.

Cunningham, R. S. 1922. The changes in the omentum of the rabbit during mild irritations; with especial reference to the specificity of the mesothelium. *Johns Hopkins Hosp. Bull.*, 33, 257.

——— 1922. On the origin of free cells of serous exudates. *Am. J. Physiol.*, 30, 1.

——— 1924. The effects of chronic irritations on the morphology of the peritoneal mesothelium. *Johns Hopkins Hosp. Bull.*, 35, 111.

Cushing, H. 1902*a*. Physiologische und anatomische Beobachtungen über den Einfluss von Hirnkompression auf den intracraniellen Kreislauf und über einige hiermit verwandte Erscheinungen. *Mitteil. a. d. Grenzgeb. d. Med. u. Chir.*, 9, 773.

——— 1902*b*. Some experimental and clinical observations concerning states of increased intracranial tension. *Am. Jour. Med. Sc.*, 124, 375.

——— 1914. Studies on cerebro-spinal fluid. *J. Med. Res.* 31, 1.

——— 1925. *Studies in intracranial physiology and surgery.* London.

Cushing, H., and Goetsch, E. 1910. Concerning the secretion of infundibular lobe of the pituitary body and its presence in the cerebrospinal fluid. *Am. J. Physiol.*, 27, 60.

Cushing, H., and Weed, L. H. 1915. Studies on the cerebrospinal fluid and its pathway: IX. Calcareous deposits in the arachnoidea. *Johns Hopkins Hosp. Bull.*, 26, 367.

Dandy, W. E. 1919. Experimental hydrocephalus. *Ann. of Surg.*, 37, 129.

Dandy, W. E., and Blackfan, K. D. 1913. An experimental and clinical study of internal hydrocephalus. *J. Am. Med. Assoc.*, 61, 2216.

——— 1914. Internal hydrocephalus. An experimental, clinical and pathological study. *Am. J. Diseases of Children*, 8, 406.

——— 1917. Internal hydrocephalus—second paper. *Ibid.*, 14, 424.

Dixon, W. E. 1923. Pituitary secretion. *J. Physiol.*, 57, 129.

Dixon, W. E., and Halliburton, W. D. 1916. The cerebrospinal fluid. IV. Circulation. *J. Physiol.*, 50, 198.

Dixon, W. E., and Marshall, F. H. A. 1924. The influence of the ovary on pituitary secretion: a probable factor in parturition. *J. Physiol.*, 59, 216.

Edinger, L. 1911. Die Ausführwege der Hypophyse. *Arch. f. mikr. Anat.*, 78, 496.

Eichhorst, A. 1875. Ueber die Entwickelung des menschlichen Rückenmarkes und seiner Formelemente. *Virchow's Archiv.*, 64, 425.

Elman, R. 1923. Spinal arachnoid granulations with especial reference to the cerebrospinal fluid. *Johns Hopkins Hosp. Bull.*, 34, 99.

Engel, E. A. 1909. Ueber die Secretionserscheinungen in den Zellen der Plexus chorioidei des Menschen. *Archiv für Zellforschung*, 2, 191.

Essick, C. R. 1920. Formation of macrophages by the cells lining the subarachnoid cavity in response to the stimulus of particulate matter. *Contrib. to Embryol. No. 42*, Carnegie Inst. Washington, Pub. No. 272, 377.

Eurich, F. W. 1898. Contribution to the comparative anatomy of the neuroglia. *J. Anat. and Physiol.*, 32, 688.

Faivre, E. 1853. *Des granulationes meningiennes.* Thèse de Paris.

Findlay, J. W. 1899. The chorioid plexuses of the lateral ventricles of the brain - their histology normal and pathological. *Brain*, 22, 161.

Flexner, S., and Amoss, H. L. 1914. Localization of the virus of epidemic poliomyelitis. *J. Exper. Med.*, 20, 262.

Francini, M. 1907. Sulla struttura e la funzione dei plessi coroidei. *Sper. Arch. di Biol.*, 61, 415.

Frazier, C. H., and Peet, M. M. 1914. Factors of influence in the origin and circulation of the cerebrospinal fluid. *Am. J. Physiol.*, 35, 268.

Fromman, C. 1864 and 1869. *Untersuchungen über die normale und pathologische Anatomie des Rückenmarkes.* Teil I, Jena, 1864; Teil II, Jena, 1869.

Fuchs, A., and Rosenthal, R. 1904. Physikalische, chemische, und cytologische und anderweitige Untersuchungen der Zerebrospinalflüssigkeit. *Wien. med. Presse,* 2082.

Galeotti, G. 1897. Studio morfologico e citologico della volta del diencefalo in alcuni vertebrata. *Riv. di Pat. Nerv. e Ment.,* **12,** 480.

Geiling, E. M. K. 1926. The pituitary body. *Physiol. Reviews,* **6,** 62.

Goldmann, E. E. 1913. *Vitalfärbung am Zentralnervensystems.* Berlin. P. 9.

Golgi, C. 1885. *Sulla fina anatomia degli organi centrali del systema nervoso.* Milano.

Haller, A. 1757. *Elementa physiologiae corporis humani.* Lausanne.

Halliburton, W. D. 1916. The possible functions of the cerebrospinal fluid. *Proc. Roy. Soc. Med.,* **10,** I.

Hannover, A. 1844. *Recherches microscopiques sur le système nerveux.* Copenhagen.

Hansen-Pruss, O. C. 1923. Meninges of birds, with a consideration of the sinus rhomboidalis. *J. Comp. Neurol.,* **36,** 193.

Harvey, S. C., and Burr, H. S. 1924. An experimental study of the origin of the meninges. *Proc. Soc. Exper. Biol. and Med.,* **22,** 52.

Herring, P. T. 1908. The histological appearance of the mammalian pituitary body. *Quart. J. Exp. Physiol.,* **1,** 121.

Hill, L. 1896. *Physiology and pathology of the cerebral circulation.* Lond.: J. & A. Churchill.

Hines, M. 1923. The hyaline bodies of the posterior lobe of the hypophysis. *Anat. Rec.,* **25,** 133.

His, W. 1865*a*. *Ueber ein perivasculäres Canalsystem in den nervösen Centralorganen.* Leipzig.

——— 1865*b*. *Die Häute und Höblen des Körpers.* Basel.

——— 1889. Die Neuroblasten und deren Entstehung im embryonalen Mark. *Arch. f. Anat. u. Physiol., Anat.* Abt. p. 249.

Houssay, B. A. 1918. *La accion fisiologica di los extractos hypofisiarios.* Buenos Aires.

Hworostuchin, W. 1911. Zur Frage über den Bau des Plexus chorioideus. *Arch. f. mikr. Anat.,* **77,** 232.

Jacob, P. 1900. Klinische und experimentelle Erfahrung über die Duralinfusion. *Deutsch. med. Wochenschr.,* **26,** 47 and 64.

Jacobson, C. 1920. A study of the haemodynamic reactions of the cerebrospinal fluid and hypophyseal extracts. *Johns Hopkins Hospital Bull.,* **31,** 185.

Kappers, A. 1924. The lumbo-sacral sinus in the spinal cord of birds and its histological constituents. *Psych. en Neurol. Bladen,* **5,** I.

——— 1925. The meninges in cyclostomes, selachians and teleosts compared with those in man. *Proceed. Royal Acad. Amsterdam,* **28.**

Key, A., and Retzius, G. 1875. *Studien in der Anatomie des Nervensystems und des Bindegewebes.* Stockholm.

Klaussner, F. 1883. Das Rückenmark des Proteus anguineus. *Abhandl. d. k. bayer. Acad. d. Wissensch.* II Cl., Bd. **14,** II Abth.

Kölliker, A. 1854. *Handbuch der Gewebelehre.* Leipzig.

1879. *Entwicklungsgeschichte des Menschen und der höheren Thiere.* Ed. 2, Leipzig.

1890. Zur feineren Anatomie des centralen Nervensystems. II. Das Rückenmark. *Ztschr. f. wiss. Zool.,* **51,** I.

Kolmer, W. 1921. Über eine eigenartige Beziehung von Wanderzellen zu den Chorioideal-plexus des Gehirns der Wirbeltiere. *Anat. Anz.,* **54,** 15.

Kolster, R. 1898. *Studien über das centrale Nervensystem. I. Ueber das Rückenmark einiger Teleostier.* Berlin.

Kubie, L. S., and Schultz, G. M. 1925. Vital and supravital studies of the cells of the cerebrospinal fluid and of the meninges in cats. *Johns Hopkins Hosp. Bull.*, **37**, 91.

Lenhossek, M. 1891. Zur Kenntnis der Neuroglia des menschlichen Rückenmarkes. *Verb. d. v. Versamml. d. Anat. Gesellsch. in München*, 193.

Lewandowsky, M. 1900. Zur Lehre von der Cerebrospinalflüssigkeit. *Zeitschr. f. klin. Med.*, **40**, 480.

Loeper, M. 1904. Sur quelques points d'histologie normale et pathologique des plexus chorioides de l'homme. *Comp. rend. Soc. de Biol.*, **56**, 1010.

Löwe, L., 1883. *Beiträge zur Anatomie und zur Entwicklungsgeschichte des Nervensystems der Säugetbiere und des Menchen.* **2**, Leipzig.

Luschka, H. 1855. *Die Adergeflechte des menschlichen Gehirns.* Berlin.

Magendie, F. 1825. *Recherches sur le liquide cephaloracbidien.* Paris.

Mauer, S., and Lewis, D. 1922. The structure and differentiation of the specific cellular elements of the pars intermedia of the hypophysis of the domestic pig. *J. Exper. Med.*, **36**, 141.

Mauthner, L. 1861. Ueber die sogenannten Bindegewebskörperchen des centralen Nervensystems. *Sitzungsber. d. math. naturw. Klasse d. Akad. Wien.*, **43**, 45.

Meek, W. J. 1907. A study of the chorioid plexus. *J. Comp. Neurol.*, **17**, 286.

Mertzbacher, L. 1905. Ueber die Morphologie und Biologie der Abräumzellen im Zentralnervensystem. *Histol. u. histolpath. Arb. über Grosshirnrinde* (Nissl-Alzheimer), **3**, 1.

Mierzejewski, J. 1872. Die Ventrikel des Gehirns. *Med. Centralblatt.*, **10**, 40.

Milian, M. 1904. Structure et relations des gaines lymphatiques perivasculaires. *Bull. et Mém. de la Soc. Anat.*, **6**, 347.

Miura, Y. 1925. Über den Gehalt der Cerebrospinalflüssigkeit an Hypophysenhinterlappensekret. *Pflüger's Archiv. f. ges. Physiol.*, **207**, 76.

Mott, F. W. 1910. The Oliver-Sharpy lectures on the cerebrospinal fluid. *Lancet*, **2**, 1 and 79.

Nañagas, J. C. 1921. Experimental studies on hydrocephalus. *Johns Hopkins Hospital Bull.*, **32**, 381.

Nansen, F. 1887. The structure and combination of the histological elements of the central nervous system. *Bergens Museum Aarsberetung.*

Pacchioni, A. 1741. *Antonie Pacchioni regiensis Medici et Anat. Romani Opera.* Editio quarta, Romae.

Pellizzi, B. 1911. Experimentelle histologische untersuchungen über die Plexus chorioidei (Adergeflechte). *Folia Neuro-Biol.*, **5**, 305.

Pettit, A., and Girard, J. 1902. *Sur la fonction sécrétoire et la morphologie des plexus choroides des ventricules lateraux du système nerveux central.* Paris.

Poirier, P., and Charpy, A. 1907. *Traité d'anatomie humaine.* Paris.

Prenant, A. 1894. Critériums histologiques pour la détermination de la partie persistante du canal épendymaire primitif. *Internat. Monatsschr. f. Anat. u. Physiol.*, **11**, 281.

Purkinje. 1836. Ueber Flimmerbewegungen im Gehirn. *Müller's Archiv f. Anat. u. Physiol.*, 289.

Quincke, H. 1872. Zur Physiologie der Cerebrospinalflüssigkeit. *Arch. f. Anat. Physiol. u. wissensch. Med., Leipzig*, 152.

Ramon y Cajal, S. 1889. Contribution all estudio de la estructura de la medula espinal. *Rev. trimestrial de histol. normal y patol.*, **1**, 79.

——— 1890. Sur l'origine et les ramifications des fibres nerveuses de la moelle embryonaire. *Anat. Anz.*, **5**, 85, 111.

Reiner, M., and Schnitzler, J. 1894. Ueber die Abflusswege des Liquor cerebrospinalis. *Centralblatt f. Physiol.*, **8**, 684.

Reiner, M., and Schnitzler, J. 1895. Zur Lehre von Hirndruck. *Wiener klin. Wochenschr.*, **8**, 371.

Reissner, E. 1864. *Der Bau des centralen Nervensystems der geschwänzten Amphibien.* Dorpat.

Renaut, J. 1882. Recherches sur les centres nerveux amyeliniques. I. La nevroglie et l'ependyme. *Arch. de physiol. normal et patbol.*, Ser. II, **9**, 593.

Retzius, G. 1893. Studen über Ependym und Neuroglia. *Biol. Untersuch.*, **5**, 9.

——— 1896. *Das Menschengebirn.* Stockholm.

Sabin, F. R. 1912. *Development of lymphatic system.* In Keibel and Mall's *Human embryology*, **2**.

——— 1923. Studies of living human blood cells. *Johns Hopkins Hospital Bull.*, **34**, 277.

Sabin, F. R., Cunningham, R. S., and Doan, C. A. 1925. Discrimination of two types of phagocytic cells in the connective tissues by the supravital technique. *Contrib. to Embryol. No. 82*, Carnegie Instit. of Washington, Pub. No. 361.

Schaper, A. 1894. Die morphologische und histologische Entwicklung des Kleinhirns der Teleostier. *Morpbol. Jabrb.*, **21**, 625.

——— 1897. Die frühesten Differenzierungsvorgänge im Centralnervensystem. Kritische Studie und Versuch einer Geschichte der Entwickelung nervöser Substanz. *Arch. f. Entwicklungsmech. d. Organ.*, **5**, 81.

Schläpfer, V. 1905. Ueber den Bau und die Funktion der Epithelzellen des Plexus chorioideus, in Beziehung zur Granulalehre und mit besonderer Berücksichtigung der vitalen Färbungsmethoden. *Ziegler's Beiträge z. allgem. Path. u. Anat.*, **7**, 101.

Sicard, J. A., and Cestan, R. 1904. Étude de la transversée meningo-radiculaire au niveau du trou de conjugation; quelques deductions cliniques. *Bull. et mém. Soc. méd. hôp. de Paris*, 3 ser. **21**, 21.

Spina, A. 1899. Experimentelle Untersuchungen über die Bildung des Liquor cerebrospinalis. *Arch. f. d. ges. Physiol.*, **76**, 204.

——— 1900–1901. Untersuchungen über die Resorption des Liquors bei normalen und erhöhten intracraniellen Drucke. I. Mitteilung. *Arch. f. d. ges. Physiol.*, 83, 120; II. Mitteilung, *Ibid.*, 415.

Sterzi, G. 1901. Ricerche intorno all anatomia comparata ed all ontogenesi delle meningi. *Atti del reale Inst. Veneto di Scienze, Lettere ed. Arti*, **60**, 1101.

——— 1902. Recherches sur l'anatomie comparée et sur l'ontogenèse des méninges. *Arch. Ital. de Biol.*, **37**, 257.

Stieda, L. 1869. Studien über das centrale Nervensystem der Wirbeltiere. *Ztschr. f. wiss. Zool.*, **20**, 273.

——— 1875. Ueber den Bau des centralen Nervensystems der Schildkröte. *Ztschr. f. wiss. Zool.*, **25**, 361.

Stilling, B. 1859. *Neue Untersuchungen über den Bau des Rückenmarkes.* Cassel.

Studnicka, F. K. 1900. Untersuchungen über den Bau des Ependyms der nervosen Centralorgane. *Anat. Hefte*, **15**, 303.

Todd. 1847. *The Cyclopaedia of Anatomy and Physiology*, Vol. **3**.

Trendelenburg, P. 1924. Die Sekretion des Hypophysenhinterlappens in die Cerebrospinalflüssigkeit. *Klin. Wochenschr.*, **3**, 777.

Van Gelderen, Chr. 1925. Ueber die Entwicklung der Hirnhäute bei Teleostiern. *Anat. Anz.*, **60**, 48.

Weed, L. H. 1914. Studies on cerebrospinal fluid. II. Theories of drainage of cerebrospinal fluid. III. The pathways of escape from the subarachnoid spaces with particular reference to the arachnoid villi. IV. The dual source of cerebrospinal fluid. *J. Med. Research*, **31**, 21.

Weed, L. H. 1917*a*. An anatomical consideration of the cerebrospinal fluid. *Anat. Rec.*, **12**, 461.

—— 1917*b*. The development of the cerebrospinal spaces in pig and in man. *Contrib. to Embryol. No. 14*, Carnegie Inst. of Washington, Pub. No. 225.

—— 1920. The cells of the arachnoid. *Johns Hopkins Hosp. Bull.*, **31**, 343.

—— 1922. The cerebrospinal fluid. *Physiol. Reviews.*, **2**, 171.

—— 1923*a*. The absorption of cerebrospinal fluid into the venous system. *Am. J. Anat.*, **31**, 191.

—— 1923*b*. The effects of hypotonic solutions upon the cell-morphology of the chorioid plexuses and central nervous system. *Ibid.*, **32**, 253.

Wegefarth, P. 1914. Studies on cerebrospinal fluid. v. The drainage of intraocular fluids. *J. Med. Research.*, **31**, 119.

Wegefarth, P., and Weed, L. H. 1914. Studies on cerebrospinal fluid. vii. The analogous processes of the cerebral and ocular fluids. *J. Med. Research.* **31**, 167.

Widal, Sicard and Ravaut. 1901. Cytologie du liquor céphalorachidien au cours de quelques processus méningés chroniques. *Soc. Méd. des hôp.*, 3 ser., **18**, 31.

Wilson, J. T. 1906. On the anatomy of the calamus region in the human bulb; with an account of an hitherto undescribed nucleus postremus. *J. Anat. and Physiol.*, **40**, 210.

Wislocki, G. B., and Putnam, T. J. 1924. Further observations on the anatomy and physiology of the areae postremae. *Anat. Rec.*, **27**, 151.

—— 1921. Absorption from the ventricles in experimentally produced internal hydrocephalus. *Am. J. Anat.* **29**, 313.

Woollard, H. H. 1924. Vital staining of the leptomeninges. *J. of Anat.*, **58**, 89.

Yoshimura, K. 1910. Das histochemische Verhalten des menschlichen Plexus chorioideus. *Arbeiten a. d. neurol. Inst. a. d. Wien. Univ.*, **18**, 1.

Ziegler, P. 1896. Ueber die Mechanik des normalen und pathologischen Hirndruckes. *Arch. f. klin. Chir.*, **53**, 75.

Section XXXII

CYTOLOGY OF THE OVUM, OVARY AND FALLOPIAN TUBE

CONTENTS

SECTION XXXII

PAGE

I. OVUM 1111
1. Dimensions of mammalian ovum 1111
2. Zona pellucida 1113
3. Formation of zona pellucida 1115
4. Special investments of rabbit's ovum 1116
5. Homologies of zona pellucida 1116
6. Golgi apparatus 1117
7. Mitochondria 1119
8. Crystalloids in ovum 1120
9. Yolk 1121
10. Formation of yolk 1121
11. Nucleus; maturation 1123
12. Degeneration of ovum within follicle 1125
13. Degeneration of unfertilized ovum in tube and uterus . . . 1127
II. MEMBRANA GRANULOSA 1128
1. Normal granulosa cell 1128
2. Granulosa in atresia 1130
III. LUTEIN CELL 1132
1. Definition and general characteristics 1132
2. Golgi apparatus 1132
3. Mitochondria 1134
4. Lipoids and neutral fat 1134
5. Pigment 1137
6. Question of specific secretory granules in corpus luteum . . 1138
7. Degeneration of granulosa lutein cell 1138
IV. THECA INTERNA 1138
V. INTERSTITIAL CELL 1141
VI. EPITHELIUM OF FALLOPIAN TUBES 1143
VII. BIBLIOGRAPHY 1145

Section XXXII

CYTOLOGY OF THE OVUM, OVARY AND FALLOPIAN TUBE

G. W. CORNER

I. THE OVUM

THE female germ cell or ovum, as it exists in the mammals, resembles the homologous cells of other animals in several particulars. Though relatively smaller than in other forms, it is still the largest cell in the body. As in other forms (although again in reduced proportion) the ovum bears a quantity of nutritive material (the yolk) serving to nourish the embryo in at least its earliest stages. Finally, the special nuclear phenomena of the ovum (synapsis, maturation) are essentially the same in mammals as in other forms.

The egg of the Eutherian mammal, as far as its special characteristics are concerned, may be described as an approximately spherical mass of cytoplasm varying according to the species from about 0.050 to about 0.150 mm. in diameter, with a nucleus which, as it approaches the mature state, becomes vesicular and peripheral. The cytoplasm contains deutoplasmic granules to an extent varying with the species. The cell when mature is contained within the zona pellucida, a protective layer, 1μ to 15μ in thickness.

The ova of the monotremes and marsupials differ somewhat from those of the Eutheria, resembling in several points the ova of birds and reptiles. The mature, unsegmented monotreme ovum is known in detail from one egg of *Platypus* (*Ornithorhynchus paradoxus*) described by Gatenby and Hill (1924); but a fairly good account may be put together from the developing ova described by Gatenby (1922) and from the numerous early segmenting ova of *Ornithorhynchus* and *Echidna*, described by the several embryologists who have studied these forms (see Wilson and Hill, 1908).

The egg is very large, as compared with the Eutherian ovum, reaching a diameter of 6.75 mm. The cytoplasmic mass, which is about 4 mm. in diameter, is not only surrounded by a zona pellucida, but by a layer of albumen more than 2 mm. thick, and by a parchment-like shell. The yolk is not homogeneous, but is differentiated into inner and outer yolk zones. In the center of the yolk there is a special area, the latebra. The marsupial ovum is best known from Hill's description of *Dasyurus* (1910) and Hartman's account of the opossum (1916). The unsegmented uterine egg of *Dasyurus* is about 32 mm. in total diameter. It is enclosed in a very thin shell membrane, within which is a laminated layer of albumen, and a zona pellucida. The uterine ovum of the opossum is slightly larger (0.4 to 0.5 mm.), chiefly due to the presence of a larger quantity of albumen. The yolk is differentiated, by a variation in its fat content, into three concentric zones; in the middle (submarginal) zone the fat is chiefly deposited.

1. *Dimensions of the mammalian ovum:*

The following table gives measurements, taken from the original reports, of the dimensions of the ova of the commonly used experimental animals,

as far as known, as well as of some which are of special interest by reason of zoological position.

TABLE I

DIMENSIONS OF MAMMALIAN OVA

Class and species	Authority. Nature of material	Diameter of cytoplasmic mass alone, in mm.	Thickness of zona, in mm.	Total diameter including zona, in mm.
UNGULATA *Sus scropha domest.* Pig	Corner and Amsbaugh, 1917 Fresh tubal ova	.....	0.010	0.160
CHEIROPTERA *Vesperugo noctula* Bat	Van der Stricht, 1909. Measured from his drawings of fixed ova	ca. 0.084–0.092	ca. 0.003	ca. 0.090–0.098
CARNIVORA *Felis domestica* Cat	Hill and Tribe, 1924. Fixed ova	0.070 × 0.082	0.0018–0.0060 increasing in tube to 0.0120	0.082 × 0.095
	Longley, 1911. Living tubal ova		0.012–0.015	0.135–0.150
Canis familiaris Dog	Van der Stricht, 1923. Measured from his drawings of fixed ova	0.0811–0.116	0.005–0.008	0.108–0.125
RODENTIA *Mus musculus, var. albinus* Albino mouse	Long and Mark, 1911. Fixed ova	mature resting ovum, 0.0655 tubal ovum, 2d. mat. div. 0.0556		
Mus norvegicus, var. albinus Albino rat	Sobotta and Burckhard, 1910. Fixed ova	mature resting ovum, 0.060–0.065 tubal ovum, 0.055–0.060	0.0015–0.0020	
Cavia cobaya Guinea pig	Rubaschkin, 1905. Fixed ova	mature ovarian ovum, 0.0550–0.0600	0.0035–0.0050	
Lepus cuniculus Rabbit	Bischoff, 1840. Fresh tubal ova			without albumen: 0.124–0.148 with albumen: up to 0.300
PRIMATES *Tarsius spectrum* Tarsier	Hubrecht, 1902. Measured from his drawings of fixed ova	ca. 0.073		
Pithecus (Macacus) rhesus Rhesus monkey	Corner, 1923. One fresh tubal ovum	0.086	.....	0.109
	Allen, personal communication. Two tubal ova	0.087 0.104	....	0.115 0.138

2. *The zona pellucida:*

The zona pellucida, which forms the external covering of the mammalian egg, is a thin shell of clear substance, approximately spherical in form (Figs. 511, 512, z.p.). To the inner surface of this layer the living cytoplasm may be closely applied, but there is no attachment between the two structures. The material of which it is formed is at least as rigid as a stiff jelly, since the tubal ovum may safely be rolled about the bottom of a dish by means of a needle, and when crushed, the zona cracks open and gapes,

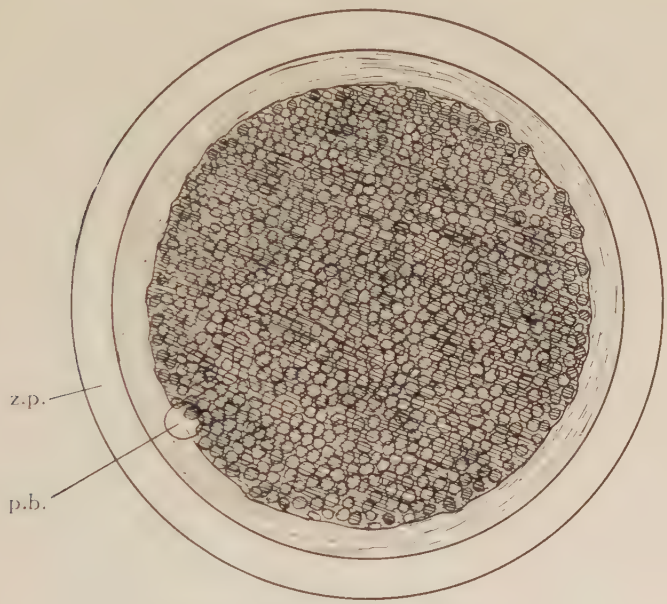


FIG. 511.—Fresh unfertilized ovum of sow, from Fallopian tube. × 400. z.p., zona pellucida; p.b., first polar body.

releasing its contents. Its chemical nature has never been worked out; judging from its behavior in the usual histological reagents, the material must be in large part of protein nature.

The older observers are almost unanimous in describing the zona pellucida as traversed by a great number of fine radial canals. Sometimes these are described as passing entirely through the zona; others trace the canals or striations into the outer portion of the zona alone. In the classical illustration of Nagel (1888) the striations are indicated more strongly in the internal part of the zona pellucida. It is usually stated that the striations are canals into which pass fine processes of the contiguous cells of the

cumulus oophorus, serving to convey nutriment to the ovum. Perhaps the most concrete of all descriptions of this type is that of the mole's egg, by Heape (1886), who shows the canals and processes with diagrammatic clearness. Fischer (1905) has given an even more complicated description of the zona pellucida; according to him three layers may be made out, an

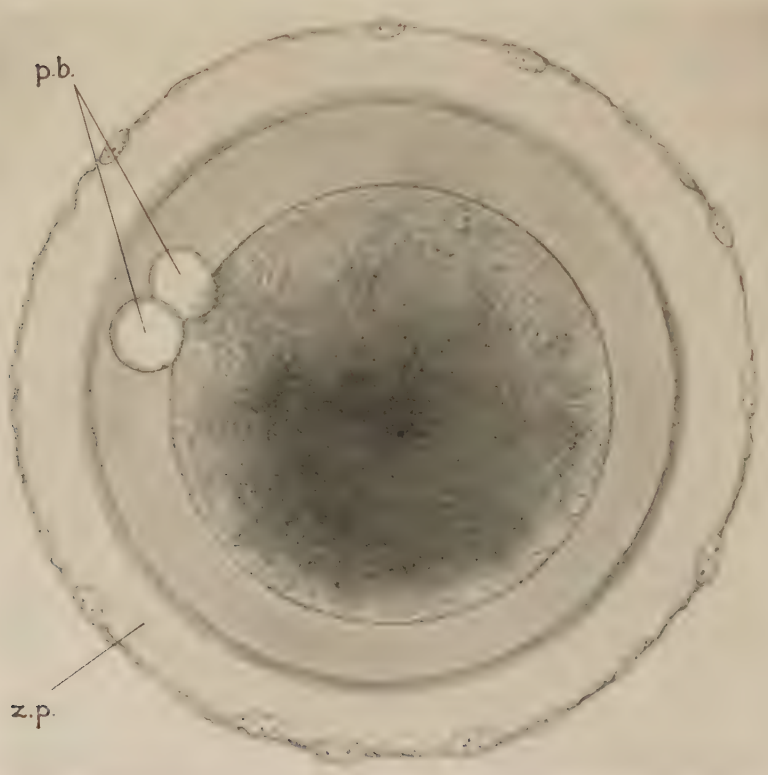


FIG. 512.—Fresh fertilized ovum of rabbit, from Fallopian tube seventeen hours after mating. The pronuclei are seen vaguely in the center. A few cells of the cumulus oophorus cling to the zona. × 400. z.p., zona pellucida; p.b., first and second polar body.

outer felt-work made up of interlacing processes of the contiguous follicle cells, an inner radial zone and an innermost homogeneous zone.

Such descriptions do not fit the actual appearances in the living ovum, as found in the Fallopian tubes of swine and the common rodents, or in thin sections of such eggs. At the most a few fine granules may be present in the zona, or at times a faint concentric layering of the hyaline material. Absolutely no radial striations can be seen, and indeed the substance of

the zona is so clear that the details of the enclosed yolk are seen as if through clear glass. When the zona is emptied of its contents and flattened by pressing a coverslip down upon the ovum, it can be studied as an exceedingly thin preparation, in which again no structure can be discerned. In stained sections of fixed material the zona pellucida may be clear, it may resolve itself into an apparent felt-work, or it may be so intensely stained as to defy analysis (as usually with iron hematoxylin) but there is no sign of radial striation.

The matter stands somewhat differently as regards the ovarian egg. When ova of the sow are removed from immature follicles of medium size and studied whole with the cells of the cumulus adherent to them, the zona pellucida then often appears to show very vague and irregular radial markings; but these would hardly draw special attention, were it not for the numerous descriptions of radial canals given by so many workers with fixed tissues.

The structure of the completed zona pellucida, therefore, requires further study, involving a comparison of the living and fixed material.

3. *Formation of the zona pellucida:*

Van Beneden (1880) who was the first to discuss the origin of the zona pellucida, supposed it to be a product of the ovum itself. This view has subsequently been upheld by a few writers, most recently by Tur (1912). Part of the evidence for this belief is drawn from cases in which two eggs lie in contact in one follicle; in these cases the zona is laid down even where each egg touches the other. In other sections, large degenerating ova have been seen which had no zona although normally placed in the follicle, and *per contra* ova are sometimes seen lying partly free of the corona radiata but still provided with a zona.

Flemming seems to have been the first to consider the zona a product of the follicular epithelium, a view which is now almost universal. Careful recent investigations by Fischer (1905) and Van der Stricht (1923) are essentially in agreement and are supported by the work of Thing (1918) who studied the analogous structure in turtles. Fischer's illustrations show the cells of the corona radiata sending out fine processes against the cytoplasm of the ripening ovum, which finally become matted together until they form a homogeneous mass. In the radial layer of the zone which he describes, the processes remain untangled, as the zona pellucida grows in thickness.

Van der Stricht (1923) considers the zona pellucida to be essentially a sort of fenestrated membrane, formed by the terminal bars or "obturating bands" which close the intercellular spaces of the follicular epithelium. Thus in tangential sections the first thin layer of the zona to be deposited is seen (in fixed tissues) as a mosaic of irregular polygonal fields. As the

deposited substance increases, the fields are narrowed until they become minute channels containing cytoplasmic processes of the follicular cells. Thing (1918), in describing a similar process in turtles' ova, illustrates the filaments of the follicle cells ending in knob-like expansions against the ovum itself, much like Heape's earlier description of the egg of *Talpa*.

It may be added that Heape did not see the radial canals in discharged (i.e., tubal) ova although he was able to depict them so clearly in the ovarian egg. It was his opinion that the processes of the follicular cells are withdrawn from their canals in the zona pellucida, during the last days before discharge of the ova, and that this withdrawal explains the fact that the canals are not clearly seen in tubal ova.

In summary, we may regard it as fairly certain that the zona pellucida is a product of the follicular cells of the cumulus oophorus, but the exact mode of its production, known only from fixed and stained material, remains uncertain.

"*Micropyle.*" It is hardly necessary to add that modern observers have not seen any orifice in the zona pellucida, adapted to permit entrance of the spermatozoon, such as described by Barry and other early writers.

4. *Special investments of the rabbit's ovum:*

Alone among the mammals, so far as investigated, the rabbit's ovum is provided during its journey through the tube with a coating of albuminous substance. By the time of arrival in the uterus this jelly has swollen until its total diameter is at least three times that of the zona pellucida.

5. *Homologies of the zona pellucida:*

The yolk-bearing cytoplasm of the mammalian ovum is not provided with any definite tunic similar to the vitelline membrane of the bird's egg. Some writers have seen, in fixed ova, a thin line at the surface of the cytoplasm, which they have been willing to call a vitelline membrane, but it is probable that this is only a surface film of the cytoplasmic colloid. Others have seen in the zona pellucida a homologue of the vitelline membrane. A structure in the egg of reptiles (i.e., turtles) has been called variously zona pellucida and vitelline membrane. The distinctive character of the mammalian zona pellucida and the fact that it is not anatomically a part of the yolk-bearing cytoplasm suffice to mark it as a special structure, serving the special function of protecting an ovum which is not to be guarded by albumen and a shell.

The appended table offers a suggestion as to the homologies of the structures surrounding the ova of vertebrates:

TABLE II

HOMOLOGIES OF STRUCTURES SURROUNDING THE OVA OF VERTEBRATES

	Birds	Amphibia	Reptiles	Monotremes	Marsupials	Eutheria
Formed in ovary	vitelline membrane	vitelline membrane				
	.....	"Chorion" or oolemma	zona pellucida	zona pellucida	zona pellucida	zona pellucida
Deposited in oviduct	albumen	egg jelly	.....	albumen	albumen	albumen (in rabbit only)
	shell membrane	.....	.....	"shell" or shell membrane	"shell" or shell membrane	
	shell	.....	shell			

6. *The Golgi apparatus:*

The Golgi apparatus of the mammalian ovum, like that of other forms (see Hirschler, 1917), is present at all stages and is carried over directly into the cells of the embryo. In young oocytes the apparatus exists in concentrated form closely applied to one pole of the nucleus, apparently lying about the idiozome (Fig. 513B). As the oocyte approaches maturity, the Golgi material is broken up into two or more masses which come to lie at the periphery of the egg (Fig. 513C), and ultimately in the stage of the germinative vesicle it exists in the form of small masses or particles distributed all about the periphery of the cytoplasm (Fig. 513D,E). Nihoul (1926) has found that at this stage it varies somewhat in appearance, according to the method of preparation. The silver-impregnation methods display it as structureless bars or anastomotic filaments which may lie so thickly as to give the impression of a fenestrated membrane. By the method of Weigl (1912), which is an osmic technique, it appears as more delicate, closely-packed filaments which may even be curved or vesiculated.

In eggs of the rabbit twelve hours after ovulation and during the period of approach of the pronuclei, the elements of the Golgi apparatus begin to gather once more near the center of the egg. According to the striking pictures of Nihoul, the greater part of the apparatus becomes concentrated about the male pronucleus; when the two pronuclei are in contact, they are both enveloped by the Golgi material, which is now so closely packed that it forms an intensely blackened corona. Nihoul did not have the good fortune to observe the first segmentation division, but he found the Golgi apparatus in the daughter-cells, again localized about the nuclei; and a similar transmission was found to occur in subsequent divisions, so that the Golgi material passes continuously from the ovum to all the cells of the embryo. It is of interest to note that the opportunity to view the Golgi apparatus in cells of such large size has led this observer to certain definite

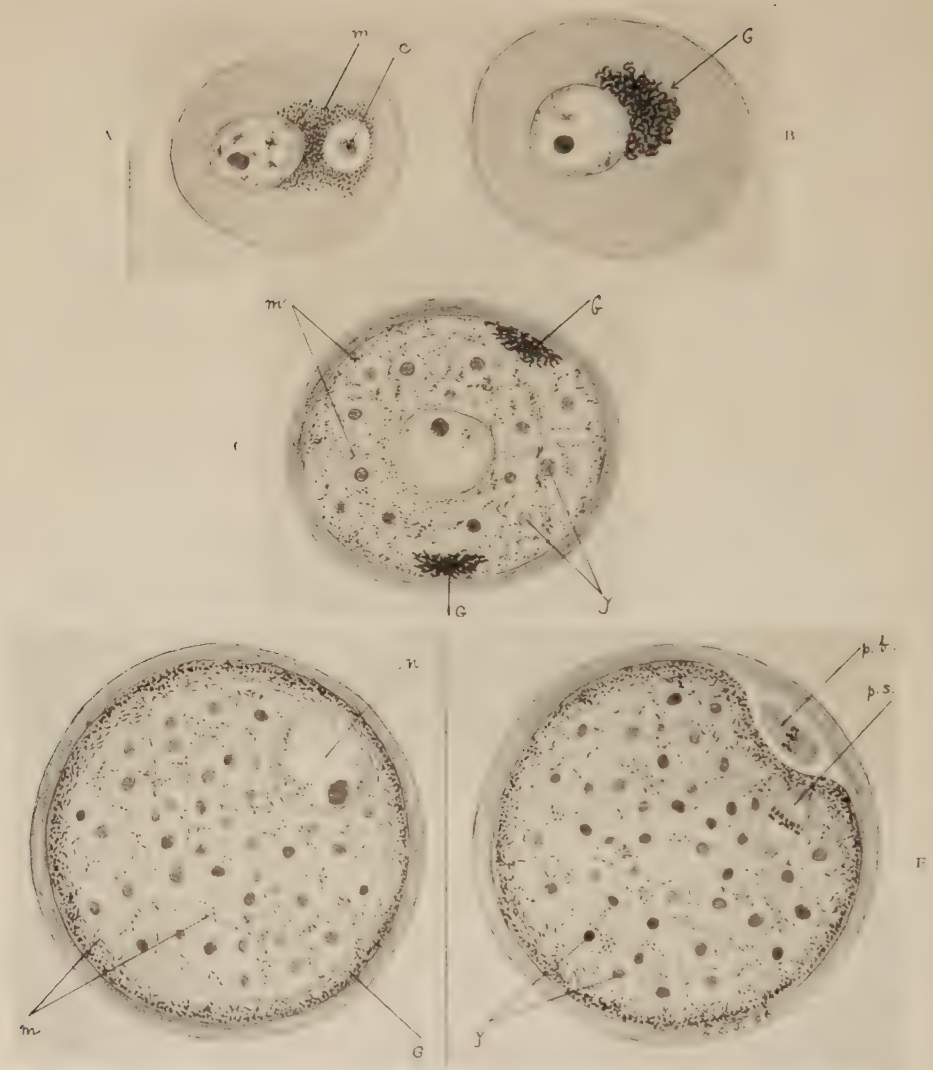


FIG. 513.—Diagrams of the cytoplasmic inclusions and maturation of the typical mammalian ovum, based chiefly upon the rabbit and guinea pig as described by Van der Stricht and Nihoul: m, mitochondria; c, centriole; G, Golgi apparatus; n, nucleus (germinative vesicle); y, yolk granules; p.b., first polar body; p.s., second polar spindle. A. Young oocyte showing mitochondria and centriolar apparatus. B. Young oocyte showing Golgi apparatus. C. Somewhat older oocyte, showing beginning of dispersion of Golgi apparatus and appearance of yolk granules. D. Advanced oocyte with germinative vesicle, illustrating peripheral position of Golgi apparatus. E. Mature ovum as at time of ovulation.

conclusions which bear on the general question as to the function of the intracellular apparatus of Golgi. For instance, the fact that the apparatus occurs (at certain stages) in the form of very slender filaments makes it difficult to believe that it is in the nature of a vacuolar apparatus. Furthermore, the idea, so often asserted, that the Golgi material participates in the formation of the yolk granules or other cytoplasmic inclusions, is answered very simply in the negative in the case of the rabbit's egg, which possesses a well-developed Golgi apparatus but is almost entirely devoid of deutoplasmic granulations. Nihoul ends his laborious task without any hypothesis as to the function of the apparatus and even without certainty that it exists in life in anything like the form which it presents in fixed and stained preparations. We shall refer later to the probable identity of this structure with the so-called yolk nucleus.

7. *Mitochondria:*

Much is known in a detailed way about the morphology of the mitochondria of the ovum at all stages of its development, but all that we have of philosophical import can be summed up in the statement that mitochondria are present in the cytoplasm of the ovum and all its precursors and that after fertilization (when they are apparently added to by mitochondrial constituents of the spermatozoon) they pass into the blastomeres and thus to all the cells of the embryo. This fact has been considered important in connection with the possibility that the mitochondria may be bearers of heredity; but up to the present there is no definite evidence that these structures are sorted or split in any regular way like the chromosomes.

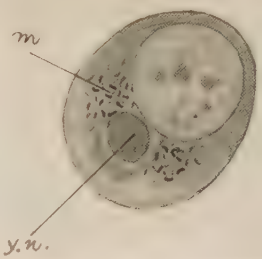


FIG. 514.—Young oocyte of guinea pig, showing mitochondria in a compact mass surrounding the "yolk-nucleus." Altmann's method after formol-bichromate fixation: m, mitochondria; y.n., "yolk-nucleus."

O. Van der Stricht's general review of the cytology of the mammalian egg (1923) forms a veritable atlas of the mitochondria at all stages of the life of the ovum. The facts described and illustrated in his numerous plates are in agreement with the reports of other workers in the same field and may frankly be accepted as representing the morphological facts as far as they can be revealed by the study of fixed tissues. During the period of growth of the oocyte the mitochondria are found to lie in a fairly dense mass concentrated about the vitelline body ("yolk nucleus") (Fig. 513A; Fig. 514). As the oocytes grow larger, there comes a period during which the mitochondria appear to move away from the centrosomal region and become scattered throughout the cytoplasm; this stage is termed by Van der Stricht "the stage of disaggregation of the mitochondrial layer" (Fig. 513C; Fig. 515).

In the final stages of growth of the oocytes, when there begins in some species to be an accumulation of deutoplasmic granules, the size of the cell is considerably augmented. At this stage the mitochondria become coarser and they begin to be condensed more

thickly in a zone about the periphery of the cell. In the final stage of growth, just before the period of maturation, there appears at the periphery of the cytoplasm (in the human ovum) a new homogeneous layer which Van der Stricht interprets as due to a liquefaction of the peripheral mitochondria. In the guinea pig and cat no such zone appears, and the mitochondrial layer remains at the periphery (Fig. 513b, E). During the period of maturation there are no further changes in these details.

Levi (1915), the only worker outside of Van der Stricht's school who has studied the mitochondria of mammalian ova, is in agreement with the general facts which the Belgian workers have given us in such detail; namely, that during the later growth and maturation of the egg the mitochondria are found chiefly in the marginal zone of the cytoplasm. After fertilization they become scattered throughout the cytoplasm, finally being carried over into the daughter-cells.

FIG. 515.—Oocyte of guinea pig, from single-layered follicle, showing mitochondria (m) in the stage of disaggregation. Altmann's method after formol-bichromate fixation.

8. *Crystalloids in the ovum:*

Athias (1915) discusses the occurrence of crystals in the cytoplasm of the mammalian ovum. Such appearances have been described in a number of species and were found by Athias in the ovarian eggs of two cercopithecoid monkeys. The structures took the form of blunt crystals, which were not perfectly homogeneous but contained areas of heightened refractility. They were not doubly refractile; were insoluble in fat solvents; and did not stain with fat-soluble stains. Athias assumes that they were of protein nature, although microchemical tests were not conclusive. One is tempted to wonder whether these crystalloids may not be due to some action of the fixing fluids; it happened that the monkey ovaries in question were fixed in Zenker's fluid and in Regaud's formol bichromate. The only point of general interest in this matter is that some writers have thought that the crystals

indicate degeneration of the egg. Athias found that in his specimens the cells containing the crystals were usually normal and that degenerating eggs tended to contain fewer crystals.

9. *The yolk:*

In the mammalian ovum yolk is present to a degree which varies greatly in different species. The eggs of rats and mice are entirely free from visible cytoplasmic inclusions, so that in the fresh ovum the nucleus is the only formed structure in a field of homogeneous cytoplasm. In the one ovum of a primate which has been studied in the fully mature stage, that of the monkey referred to on page 1125, the cytoplasm was so finely granular as to be almost homogeneous in appearance, even under the compound microscope. The egg of the rabbit contains a few yolk bodies, but not enough to obscure the nucleus when seen fresh (Fig. 512). The guinea pig's ovum contains more yolk than the rabbit's, in the form of small, rather uniform spherules. The ova of the cat and dog contain larger amounts of yolk granules, those of the cat being characteristically variable in size; in the dog the granules are so numerous as to lie almost in contact throughout the cytoplasm. Finally, the ova of the domestic pig are so heavily laden with globules (3μ to 5μ in diameter) that the cytoplasm is almost completely opaque to transmitted light, and the egg is therefore easily seen under the microscope, in washings from the Fallopian tube, as a dark circle surrounded by the clear zona pellucida (Fig. 511).

It appears that the human ovum, like that of the monkey, is almost devoid of yolk granules, but we have not enough information about normal eggs in late stages to permit a very positive statement. One is tempted to conjecture that the large amount of yolk in the domestic pig is connected with the fact that the embryos of this species are not attached to the uterus until a considerably later stage in their development than those of the rat, guinea pig and rabbit. It may well be that such embryos require a store of nutriment in the egg to tide them over the period before they begin to receive food materials from the mother.

The chemical nature of the yolk globules in mammals has not been well worked out, but they are largely of lipoidal character. It is known that they are generally stainable with Sudan III and with osmium tetroxide. A few writers have mentioned yolk granules which do not take fat stains.

10. *Formation of the yolk (the "yolk nucleus"):*

There are three extant theories as to the mechanism of yolk formation in mammals. In that which we owe to Hertwig, the deutoplasmic granules are thought to be laid down by chromidial material extruded from the nucleus. This theory has in its favor nothing more than deductions from fixed preparations. In a second theory it is assumed that the lipoids of the yolk pass bodily into the cell by secretion from the cells of the Graafian follicle and especially from the corona radiata. Russo (1912) asserts that he has been able

to increase the amount of mitochondria and ultimately the amount of deutoplasmic granules in the egg of the rabbit by injecting the animal with lecithin. During the process of storage of the injected material he finds an increase of granules in the follicular cells about the ovum; and, moreover, other granules thus produced can be seen occupying the channels in the zona pellucida with which the follicular cells are continuous, and also in the perivitelline space. In animals which have been starved the mitochondria become vague and even disappear (Russo, 1910). During the process of formation of yolk in normal eggs Russo finds some of the mitochondria arranged about vacuoles in the cytoplasm, and he interprets this picture as indicating a transformation of mitochondria into yolk.

Thirdly, the workers of the Ghent School, whose work has been summed up by O. Van der Stricht (1923), have interpreted their preparations as demonstrating the formation of yolk granules through deposition by the mitochondria, or at least by transformation of a vitellogenous zone about which, in early oocytes, the mitochondria are concentrated. In these young eggs there exists next to the nucleus a specialized cytoplasmic area which typically consists of a central body surrounded by a clear zone, which is in turn surrounded by a spherical or crescentic mass of densely packed mitochondria (Figs. 513A; 514 y.n.). This region, which has long been known under various names, such as Balbiani's body, yolk nucleus, etc., is called by Van der Stricht "*couche vitellogène*." As the oocyte grows older and larger, the mitochondria of the *couche vitellogène* are dispersed throughout the cytoplasm until ultimately a marginal mitochondrial zone is formed, as described in a previous section. During the process of dispersal, vacuoles and small granules appear in the cytoplasm in the intermediate region between the nucleus and the marginal mitochondrial zone, and these develop into the characteristic yolk granules. At the moment of disaggregation of the mitochondrial layer of the *couche vitellogène*, changes also take place in the yolk nucleus (R. Van der Stricht, 1911). This structure divides into two or three smaller bodies, and these wander toward the periphery of the ovum, where they usually divide again, sometimes forming as many as ten yolk nuclei in one ovum.

Sjövall (1906) has pointed out the striking resemblance between the behavior of the yolk nucleus and the internal reticular apparatus of Golgi. The perinuclear position of the yolk nucleus, its division and the ultimate dispersion of its remnants about the periphery of the cells are phenomena so characteristic that it is difficult to accept the similar behavior of the Golgi net as a mere coincidence. This resemblance has only been strengthened by the work of R. Van der Stricht and of Nihoul some years after Sjövall's conjecture. It is to be hoped that the two appearances may be investigated in the same material. At present we do not know the exact relations between the mitochondria and the Golgi net in the young oocyte. Monterosso (1915) has described alterations in the mitochondria of growing mammalian ova, coincident with the formation of deutoplasmic granules, which he interprets as indicating the formation of yolk by the mitochondria.

The present writer can sum up the question of the origin of the yolk not from the results of personal research but only as a referee of the works of others. The same question, as it comes up in connection with the much more extensive yolk formation of lower forms, has long been discussed with equally inconclusive results. It is merely one phase of the general problem of the transportation and deposition of fats in the animal body. The best biochemical opinion is that we still know nothing as to the mechanism by which fat and other similar substances are transported through animal membranes (see Bloor, 1922). The microscope has failed us completely with

respect to the passage of fats through intestinal epithelium and their reconstitution in the portal blood; the careful cytological studies on the egg yolk, which we have quoted, fail us in the same way. After all, what they tell us is merely that fats are laid down inside the egg, where previously they did not exist. The special behavior of the mitochondria and the Golgi net in the egg and their apparent physical association with the yolk granules may or may not be significant. It seems highly probable that the lipoid material of the yolk passes from the blood stream through the follicular wall and into the egg in some soluble form, perhaps as a phosphatized fat; it is unlikely, therefore, that the whole course of its history will ever be traced by the microscope alone.

11. *The nucleus; maturation:*

The nucleus of the young oocyte occupies a central position in the cell, and even in the earlier stages it is characterized by fairly large size and a relatively vesicular structure, with its strands of chromatin lying close to the nuclear membrane and stretched across the nucleus as a light network. At some period in the growth of the oocyte, not exactly to be defined, the nucleus moves toward the periphery of the cell and becomes even more vesicular than before, until at last it forms a tensely swollen sphere with a prominent nucleolus and with its cytoplasm largely condensed against the nuclear membrane. In this stage (Fig. 513D) it is called the germinative vesicle.

As in other divisions of the animal kingdom, the mammalian ovum is prepared for fertilization by undergoing certain nuclear changes which, taken together, form the process of maturation. Maturation has been studied in a more or less complete way in representatives of four orders among the twelve orders of vertebrates, namely, rodents, marsupials, carnivores and cheiroptera. We have also some information from representatives of the insectivores, the ungulates and the primates. Readers who wish to follow detailed accounts of maturation in individual species will obtain the literature from the papers of Van der Stricht (1923), Athias (1910), Rubaschkin (1905) and Long and Mark (1911). All this detailed work has demonstrated a remarkable unity in the process of maturation as it occurs even in widely separated mammals. With one exception (the dog, Van der Stricht, 1908), in all accurately known cases the maturing ovum while in the follicle forms and extrudes the first polar body and is discharged into the Fallopian tube with the second polar spindle formed or forming (Fig. 513E). The spermatozoon enters the ovum at this stage, and the second polar spindle is extruded during the passage of the male pronucleus toward the female pronucleus.

The process of maturation appears to begin, in normal eggs, in normal follicles which are destined to rupture only a short time before discharge

of the egg. In the rabbit, for instance,in which maturation of the ovum and ovulation occur only after mating, we know that the process of maturation up to the formation of the second polar spindle occupies but ten hours. Long and Mark (1910), with very carefully timed material from the white mouse, state that maturation in this species requires something between four and fifteen hours. In a number of other animals it can be deduced from the extant data that the ovum passes from the stage of the germinative vesicle to that of the second spindle within not more than a day or two (guinea pig, cat, domestic pig, etc.). The fact that maturation occurs speedily and immediately before ovulation does not seem to be widely known except among specialists in this field, but it must be taken into consideration in the discussion of difficult special cases like that of the human species. One of the reasons why the painstaking work of Thomson (1919), on the human ovum, has not been accepted is that this author has described apparent maturation in more than one ovum in the same ovary and in eggs of diverse size, some of them in small follicles. It is fairly safe to assume that when the maturation of the human egg is certainly known, it will prove to resemble in its chronology the same process in other animals.

The process of maturation is begun by the disappearance of the nuclear membrane and by a practically simultaneous condensation of the chromatin into small, rounded, compact masses which gradually arrange themselves into short, thick rods or crosses situated on the equator of a mitotic spindle. The spindle may be either oblique or perpendicular to the surface of the egg, according to the species. In the mouse Long and Mark have always found the polar spindle to be oblique to the surface at the moment of abstriction of the polar body. After the detachment of the polar globule, the second polar spindle rapidly forms and proceeds to the metaphase. Van der Stricht (1923) has called attention to the fact that the chromosomes of the second spindle differ from those of the first in all mammals which he has studied; in the second spindle each chromosomal mass consists of two small curved rods. Long and Mark have shown the same thing in describing the chromosomes of the first spindle (in the mouse) as tetrads, those of the second spindle as dyads. There is some divergence of opinion regarding the centrosomes in the maturation divisions. Long and Mark never saw centrosomes in the mouse's egg, but found the central area of the asters occupied by large amorphous masses of chromatic material which they called "circumpolar bodies." Van der Strieht and his pupils have observed centrosomes or central corpuscles in both maturation spindles, in the bat, white mouse, cat and dog, but never in the guinea pig, and the absence of these bodies in the guinea pig is confirmed by Moore and Tozer (1908).

The first polar body may suffer one of several fates. Perhaps the commonest thing is for it to remain undivided by the side of the second polar body, but in some mammals (i.e., the white mouse) it often degenerates

soon after formation. This fact led to a supposition on the part of some workers that the mouse produces only one polar body, but the investigations of Long and Mark have demonstrated that all fertilized eggs form two polar cells. In mammals the chromatin of the first polar body often forms a mitotic spindle and occasionally it divides (Moore and Tozer), thus reaching a stage which is usual in some invertebrates, in which all four daughter cells of the meiotic division are present (i.e., the ovum and three polar bodies).

Van der Stricht (1908) has shown that the dog forms an exception to the rule among mammalia, for its ovum is discharged from the ovary with its nuclear material still in the form of a germinative vesicle, and is inseminated in this state; thereafter the two polar bodies are formed and extruded in succession.

Among the primates little or nothing is known about maturation. We have a note about *Tarsius spectrum* by Hubrecht (1902) and one egg of *Macacus rhesus* has been described by the writer (Corner, 1923). In these species it appears that the egg is discharged from the ovary in the stage of the second polar spindle. The only attempt to study maturation of the human ovum is that of Thomson (1919), who believes that the second polar body is extruded before the oocyte has left the Graafian follicle, and therefore that maturation is complete before semination. In view of the discrepancy between this finding and the common rule in mammals, Thomson's results have been subjected to much critical examination, and it must be stated that in the opinion of his critics the pictures which he presents can be explained by a fact which we have, already mentioned namely, that appearances closely simulating maturation may occur in eggs which are beginning to degenerate, even in follicles which seem normal.

The mammalian egg has not proved thus far to be very useful material for chromosome counts, probably because of the fact that the cells must be fixed as they lie in the ovary or in the tube, and also because the greatly improved modern methods of fixing mammalian chromosomes, which have made possible the accurate counting of these structures in the testes, have not been applied to the ovum.

12. *Degeneration of the ovum within the follicle:*

It is well known that a large proportion of the oocytes of the adult animal do not reach maturity, but degenerate in the follicle. In some cases the ovum shows signs of degeneration before the other elements of the follicle are altered; but often the ovum remains unchanged until its investments have degenerated, so that the ovum may be for a time the least abnormal structure in an atretic follicle. These changes have been studied more fully in the guinea pig than in other forms, but they may be observed in all mammals. One of the characteristic changes is the appearance of a caryocinetic

figure (Fig. 516), followed usually by fragmentation of the cytoplasm. Some of the older writers considered this the expression of an abnormal segmentation of the ovum, but recent opinion, following Rubaschkin (1907), has assumed that the spindle which forms is a polar spindle and, therefore, that the mitotic changes indicate an abortive maturation. Branca (1926) believes that both types of division occur. The question has been reopened to a certain extent by the observations of Leo Loeb (1911), who has found in the follicles of young guinea pigs a kind of proliferation of the degenerating

Fig. 516.—Above, atretic follicle of monkey (Macacus rhesus) containing an ovum in a state closely imitating normal maturation. × 144. Below, the same ovum enlarged, showing a first polar body and a second spindle. × 640.

ovum, leading to the production of complex cell masses which bear a great resemblance to embryonic tissue, which he interprets as in the nature of parthenogenesis. Other writers, including especially Stockard and Papanicolaou (1917), have not found such elaborate changes of the ova of adult guinea pigs, but it is not uncommon in this species to see groups of pseudoblastomeres to the number of two, four, eight or even more.

A number of writers have called attention to the appearance of invading cells within the zona pellucida of the degenerating eggs which have been described as carrying on phagocytic destruction of the ovum. Without special technical methods it is difficult to be certain whether or not a given nucleated mass of cytoplasm is an invading cell or a fragment of the degen-

erating egg, and it is even more difficult to prove it phagocytic. One or two recent writers (Asami, 1920, and Clark, 1923), are in disagreement on this point, the latter denying all phagocytosis of the egg, the former denying at least phagocytic destruction of the healthy ovum. On the other hand, in certain preparations from vitally stained animals, described without illustration by Evans (1916), but demonstrated a number of years ago to the writer, the dye was found to be accumulated in cell-like bodies within the zona pellucida. The zona pellucida is relatively resistant to degeneration, and usually remains, although it may become greatly distorted, as the last visible remnant of the ovum in an atretic follicle.

13. *Degeneration of the unfertilized ovum in the tube and uterus:*

The unfertilized ova of animals which ovulate spontaneously begin after a few days to degenerate in the Fallopian tube and complete their dissolution in the uterus. According to Charlton (1917), the eggs of the albino mouse begin to degenerate during the second day after discharge from the ovary, showing as the first change a breakdown of the spindle of the first polar body and a resolution of the polar nucleus into one or more resting nuclei. The fibers of the second maturation spindle also disappear and the egg nucleus may also be resolved into smaller nuclei. The cytoplasm stains more deeply at this time than in normal eggs and ultimately becomes fragmented into a number of small masses which may be so uniform as to resemble the morula stage of the embryo. In the mouse the zona pellucida nearly always persists until the cytoplasm is completely fragmented. Charlton states that these degenerating ova are invaded by phagocytic cells which he takes to be polymorphonuclear leucocytes, but one is not entirely convinced by this interpretation, as illustrated in his drawing. The final dissolution of the egg is completed on the fourth day.

The author has seen hundreds of disintegrating unfertilized eggs of the sow, obtained by washing the uterine cavity between the third and the seventh or eighth day after ovulation. The process of degeneration is much the same as described by Charlton, as far as can be made out by examination of the whole egg. The pseudo-blastomeres produced by the division of the cytoplasm are seldom uniform in size; often there is one large mass and a number of smaller ones. In the last stages of the process the cytoplasm is broken up into numerous very small granules and the zona pellucida becomes swollen, loses its refractility and becomes flattened until it has a distinctly lenticular form. After a week the vanishing ovum consists only of a few fine granules in an almost invisible disc of flattened zona. The only primate ovum which has ever been seen in this stage is one of *Macacus rhesus* described and illustrated by the author (Corner, 1923), which was taken from the uterine cavity a few days after ovulation and which resembled very closely degenerating ova of the sow as just described.

A few fertilized ova of the rabbit, made to degenerate in the Fallopian tube by experimental lesions of the ovary, showed almost complete dissolution of the cytoplasm, so that the ova consisted of practically empty zonae.

II. THE MEMBRANA GRANULOSA

1. *The normal granulosa cell:*

The cells of the membrana granulosa, lining the Graafian follicle, do not present any cytological features of peculiar interest in themselves until the time of their transformation into lutein cells or their degeneration in atresia.

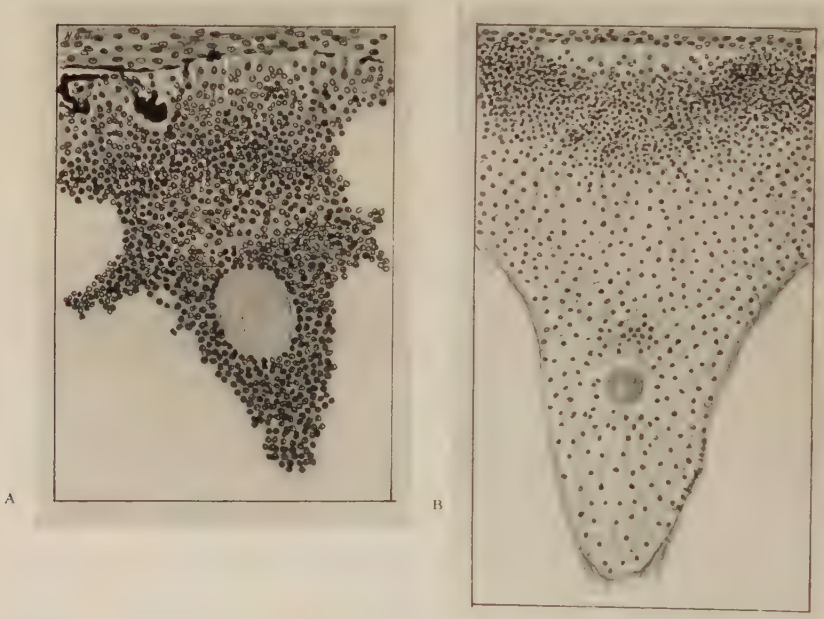


FIG. 517. — A, cumulus öophorus of a large follicle not yet mature. × 112. B, cumulus of follicle containing mature egg, during estrus, showing alteration of the cells of the cumulus. × 48.

They have been shown (Levi, 1913) to contain mitochondria in the form of long and intricately woven filaments. The presence of a small Golgi net in the cells of the granulosa has recently been demonstrated by Solomons and Gatenby (1924). In the basal layer of the granulosa the Golgi material is located at the side of the nucleus farthest from the membrana propria; in the over-lying granulosa cells no special orientation has been made out. When the granulosa cells are converted into lutein cells, the Golgi net is very much enlarged. In form the granulosa cells are polyhedral, owing to the dense manner in which they are packed together, except those cells which are immediately about the ovum (the so-called corona radiata) and the

basal layer next to the theca interna (Fig. 517A). In these two places, at which the granulosa cells abut on extraneous surfaces, they assume a roughly columnar form. In large follicles of a number of species, the granulosa cells contain a few very small granules of neutral fat, which are more numerous in the basal layer.

The granulosa of some species contains peculiar bodies which for want of agreement as to their nature are usually called the bodies of Call and Exner; they are well seen in the rabbit and in some monkeys and also (judging from the detailed description of Thomson, 1919) in the human follicle. They consist (Fig. 518) of spherical bodies, ranging in size from a few micra to as much as a hundred micra, about which the granulosa cells tend to assume a columnar arrangement, resembling the corona radiata about the ovum; and, in fact, structures of this sort may present a considerable resemblance to the ovum. The bodies themselves consist of a mass of homogeneous or granular material, staining faintly with acid dyes and sometimes containing at the core a granule or two of denser material staining with nuclear dyes. These bodies are not present in all follicles, but when present at all, there are usually several in one follicle. A number of hypotheses have been made to explain their nature. Honoré (1900) considers them to represent the first stages of secretion of the follicular fluid, and this view is supported by Thomson (1919). Benckiser (1884), who saw them in the pig, inclined to view them as mere degeneration products of dead granulosa cells. They have even been alleged to be masses of an ovarian hormone. No one appears to have suggested the possibility that these bodies may consist of the same material as the zona pellucida secreted upon casual granules or detritus by the nearest cells of the granulosa; they seem to take certain dyes, notably aniline blue, much like the zona pellucida. The suggestion that they are actually the precursors of the follicular fluid seems to me to be weakened by the fact that their size and number are far from constant in follicles of comparable size, even in the same species. It must be admitted, on the whole, that we have no definite information regarding the manner of formation of either the follicular fluid or the zona pellucida.

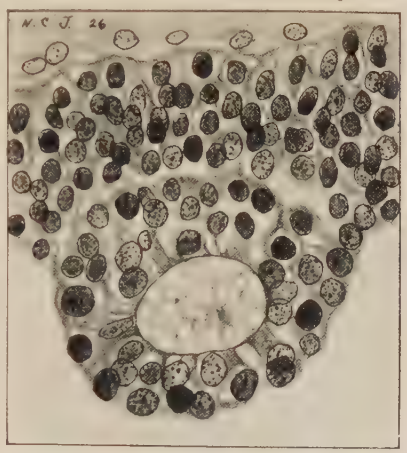


FIG. 518.—Part of granulosa of small follicle, rabbit, showing a "Call and Exner body." × 900.

There is a special modification of those granulosa cells forming the cumulus oophorus, which takes place during the few days immediately preceding rupture of the follicle. The cells of this region become very much

swollen by vacuolization of the cytoplasm so that the nuclei lie farther apart from each other, and the cells are finally held together only by strands of cytoplasm. The cells which form the corona radiata still hold together but are drawn out into long radial strands (Fig. 517B). This change takes place throughout the whole of the cumulus, leaving an unchanged zone of the granulosa at the base only, so that when the follicle ruptures and collapses the altered cumulus oophorus crumbles away, and the egg is thus cut off from the follicular wall. In species with relatively small follicles in which the ova do not lie in cumuli but are bound to the wall of the follicle by retinacula, a similar change takes place in the retinaculum. The altered cells disintegrate and fall away from the ovum as it travels through the Fallopian tube.

After discharge of the egg the granulosa cells, in all accurately known species (including man), remain in their original position in the collapsed follicle. Up to the moment of rupture a few mitotic figures are seen in the granulosa, but thereafter for some unexplained reason cell division does not occur, except perhaps as an occasional rarity. The granulosa cells immediately begin to swell, passing within a few days (in the pig, for instance) from a diameter of 8μ or 12μ to dimensions of 30μ to 35μ. The nuclei become larger and more vesicular and the mitochondria (Levi, 1913) change from a filamentous to a granular form. The cytoplasm shows a very marked increase in the amount of neutral fat. As we have seen, the granulosa cells contain very little osmium-staining material, but the corpus luteum of the pig, the mouse, the rat, the cat and other species is heavily laden, after a week, with osmium-blackening granules and globules. In the pig there is also an increase in amount of other lipoids present in the young lutein cells, so that after certain common fixatives these cells contain an extraordinarily complex system of vacuoles, which will be discussed below (page 1133).

2. *The granulosa in atresia:*

The histological problem offered by atresia of the Graafian follicle is complicated by the existence of various types of follicular destruction and metaplasia in different mammals. On one hand, as in the dormouse, *Eliomys quercinus* (Vellosa de Pinho, 1925) and in some catarrhine monkeys (unpublished observations of a colleague) the granulosa cells may persist and attain a complete resemblance to interstitial cells. At the other extreme the process of atresia is characterized by a rapid chromatolytic destruction of all the granulosa cells. This is the usual fate of the granulosa in degenerating follicles of the commoner species of laboratory animals. The exact details of the chromatolytic process have recently been subjected to study by Salazar (1919) who makes the interesting statement that the sudden death or sideration of the nucleus usually sets in during one stage or another of mitotic division. The chromosomes lose their independence

and coalesce into one or more chromatic masses which gradually become rounded. Thereafter the chromatin retracts until it forms a mere shell about a hyaline spherule at the site of the former nuclear material; and finally the cell disappears by plasmorrhexis. Although according to Salazar the onset of nuclear decay most commonly occurs in dividing cells, it may also take place in resting nuclei. True pycnosis and caryorrhexis are said to be rare, but Salazar describes somewhat similar forms of nuclear destruction under the terms "chromatolysis by margination" and "concentric chromatolysis," in which the chromatin gathers about the nuclear margin, in the former variety, or in more or less concentric layers about a hyaline core, in the second variety. There is also a sort of direct nuclear atrophy which has been described by one or two writers. If Salazar's view that the onset of destructive changes usually occurs in dividing nuclei is valid, an interesting question is raised as to whether the abortive mitosis is a result of the degenerative process affecting the follicle (just as we suppose the simulated maturation of decadent ova to be the result of degeneration) or whether normal mitosis of the granulosa, which would have been of constructive character, is merely overwhelmed by the onset of atresia. Salazar leans to the latter view, but he has asserted (1924*b*) that impending atresia may be detected in otherwise normal follicles by the onset of such abortive mitoses.

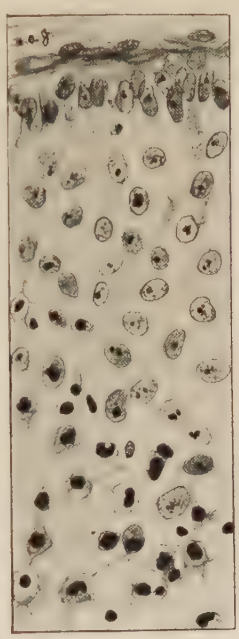


FIG. 519.—Granulosa of atretic follicle of monkey (Macacus rhesus). Showing nuclear changes in degenerating cells.

As shown in the illustration (Fig. 519) the nuclear forms produced by chromatolysis are diverse and often bizarre and the detached cells of the granulosa may finally present a considerable resemblance to polymorphonuclear leucocytes and to other wandering cells. Salazar (1922) is inclined to think that most of the cases of invasion of atretic follicles by leucocytes described in the literature are based upon misinterpretation of such resemblances. It must be admitted that in late stages of atresia a few leucocytes enter the follicular cavity. The problem is complicated by the fact, first described by Evans (1916) and confirmed by Borell (1919), that during atresia the basement membrane of the follicle, which normally forms a complete barrier to the passage of particulate matter such as the vital azo dyes, suddenly becomes pervious to these substances. In the ovaries of a rabbit, stained vitally with trypan blue or carmine, the degenerating granulosa cells of atretic follicles are filled with ingested masses of the dye. Evans states that at a somewhat later stage of atresia the follicle is invaded by true macrophages (clasmatocytes), which of course

also bear accumulations of vital stain. Since it happens that practically all the cytological descriptions of chromatolysis and phagocytosis in atresia have been worked out in the rabbit and rat, studies of this extraordinary destructive process in other forms, and especially outside the rodents, are much to be desired. The use of vital stains promises to add greatly to the progress of this difficult subject.

III. THE LUTEIN CELL

1. *Definition and general characteristics:*

In discussing the corpus luteum the author assumes the correctness of the view, now almost universally held (see Corner, 1919, for literature) that the large epithelioid cells of the corpus luteum, the so-called "lutein cells," are produced by the hypertrophy of the granulosa cells. As a literal cytological descriptive name, "lutein cell" is incorrect, as we shall see, since few animals actually have pigmented corpora, and even in these the pigment is not wholly in the cells in question; but the term is honored by usage and cannot be dropped. As used in this chapter the word "lutein cell," therefore, refers to the typical large cells which are derived from the granulosa.

If the contention of Van der Stricht (1912), Corner (1919), and others is correct, that at least in some species the theca interna cells persist in the corpus luteum either as special elements, as in the pig, or indistinguishably from the granulosa derivatives, as described in the bat by Van der Stricht, then it becomes necessary to discriminate between the two strains of cells of the corpus luteum. For this reason I have used once or twice the term "granulosa-lutein cells" as opposed to "theca-lutein cells." The granulosa-lutein cell is a large rounded or polyhedral cell, reaching in some species a diameter of 60μ. It possesses a large vesicular nucleus usually provided with one rather prominent nucleolus. Owing to the usual presence of fatty inclusions in the peripheral portion of the cell, two cytoplasmic zones may be made out (at least at certain stages), an inner or perinuclear homogeneous endoplasm and an outer vacuolated exoplasm.

2. *Golgi apparatus:*

The Golgi apparatus exists in the large cells of the corpus luteum, as in practically all the cells of the body (Fig. 520). In the fully developed lutein cells it forms a large perinuclear net with wide meshes and relatively slender trabeculae. In cells which have been distorted in form the Golgi net may be elongated or otherwise modified in shape; it is not always seen in the form of a continuous network but may be fragmented. Riquier (1910) has followed its alterations in the degenerating corpus luteum of the cow and finds that as the cells grow smaller, the Golgi apparatus tends to shrink and to

leave the perinuclear region, finally coming to lie near one of the poles of the cell. In the last stages of degeneration the apparatus is seen as an angular clumped body of indefinite structure.

In a monograph of the present writer (Corner, 1915) there are certain misleading remarks about the Golgi apparatus in the lutein cells which may well be corrected in this place. When the corpus luteum of the sow during the first half of the ovulation period or the first half of pregnancy is fixed in aqueous coagulants like formol, the oily lipoids of the lutein cells tend to round up about the globules of the neutral fat in the cytoplasm. Thereafter, as the cell proteins are coagulated, the periphery of the cell becomes filled with shrinkage spaces, within each of which is a sphere of protein-lipoid

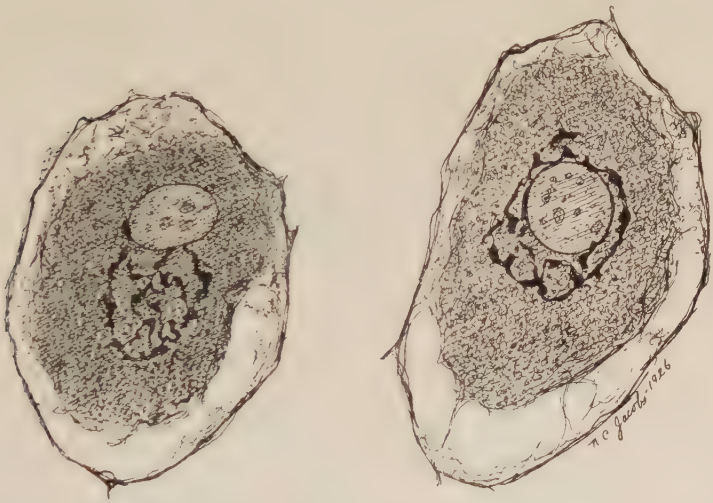


FIG. 520.—Golgi apparatus in lutein cells of pregnant sow. Silver impregnation after uranium nitrate.

material inclosing a globule of neutral fat, the whole forming a striking and characteristic picture (Fig. 522A) (see p. 1134). At the time these appearances were first seen, the trophospongium theory of Holmgren was actively afloat, and I was led to suppose that the spaces just described were in fact a peculiar modification of Holmgren's canalicular system, which I also supposed (as did many others) was homologous with the Golgi apparatus. These views, expressed as they were with some reservation, were not discouraged by two cytologists of international reputation to whom the preparations were shown; but later, having made the preparations from which the accompanying illustration is drawn, a genuine Golgi net of the usual type was found, and the peripheral canals were discovered to be an artefact

of fixation. This error will serve at least to illustrate the dangers of comparison between little-known cytoplasmic structures on the basis of single technical procedures.

3. *Mitochondria:*

The existence of typical mitochondria in the lutein cells (Fig. 521) is easily observed in fresh tissue with the usual Janus green technique. Levi (1913) has shown (in the guinea pig and several species of bats) that the characteristically long and sinuous mitochondria of the follicular epithelial cells are promptly altered into short rods in the early lutein cells. Toward the end of the period of gestation, it is stated that the mitochondria are less clearly demonstrable. They do not have any fixed morphological relation to the fat droplets.

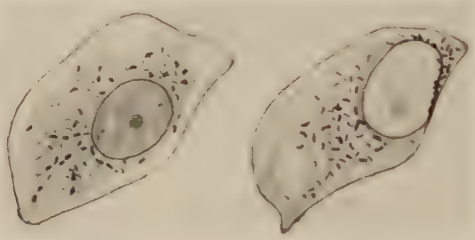


FIG. 521.—Lutein cells of guinea pig, showing mitochondria. Altmann's stain after formol-bichromate fixation.

4. *Lipoids and neutral fat:*

The lutein cell is characteristically laden with fatty material. We have already seen that there is a small amount of osmium-staining material in the cells of the granulosa of the follicle. As these cells are converted into lutein cells, the amount of fat gradually increases, until by the time the corpus luteum is a solid structure the cell in many species is completely laden with fat granules. The only species, so far as I know, in which the history of the lipoid inclusions has been followed to the end are the pig (Corner, 1915, 1919, 1921) and the rat (Long and Evans, 1922). In the pig the lipoidal inclusions of the cell vary in so definite a manner that the successive stages of the life cycle of the corpus luteum are easily distinguished under the microscope. During the first few weeks of pregnancy not only is there a large number of globules, giving the reaction of neutral fat, in the cytoplasm; but there appears to be a high content of lipoids, presumably of phosphatide nature, diffusely throughout the cytoplasm. When such cells are fixed in aqueous fluids, we obtain the oily droplets described in the preceding paragraph (Fig. 522A), which are soluble in alcohol of 60 per cent or stronger, in ether, chloroform, acetone, benzene and xylene; they stain blue with Nile blue sulphate, pale brick red with neutral red, yellowish with

osmium tetroxide (changing to gray in alcohol), are positive with Ciaccio's method and give a brown lake with Weigert's hematoxylin; they are not anisotropic.

Alcoholic extracts of the corpora lutea of swine contain, mixed with neutral fat, a fat-like substance of pasty consistence which is yellowish in color, readily becoming brown in the air, and presenting a characteristic "greasy" odor. It gives microchemical reactions exactly like those mentioned before for the droplets in the cells, except that as it rounds into spheres when in contact with water, the spheres are momentarily anisotropic.

The tests cited indicate that the substance, thus revealed by a fortunate artefact of fixation, is probably a lipoid or lipoid mixture of the phosphatide series, containing perhaps a fatty acid (suggested by the partial reduction of osmium tetroxide). Although Fenger (1914) has shown the presence of

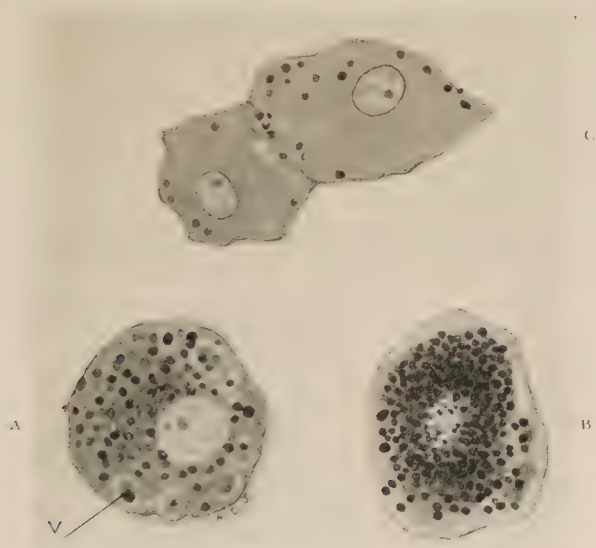


FIG. 522.—Lutein cells of pregnant sow, stained with osmium tetroxide to demonstrate fat. × 650. A, early pregnancy (embryos 20 mm.), osmium tetroxide after formol, showing ring-shaped artefacts (v). B, the same, fixed and stained with osmium tetroxide. C, late pregnancy (fetuses 230 mm.) fixed and stained with osmium tetroxide.

large amounts of phosphatides in the corpora lutea of cows, the microscopic appearances described above cannot be found in the bovine species. Perhaps the lipoids are here in a less oily condition, not rounding up in water so readily; or they are dissolved in other cell lipoids which themselves do not produce the peculiar spheres which have been mentioned. In the dog the appearances are exactly as in swine, and certain bodies seen by Cohn (1903) in the lutein cells of rabbits are almost certainly due to the same cause. The lipoid made visible by this method is present only during the earlier

part of pregnancy, and cannot be detected after about the middle of the term.

The granules of neutral fat also diminish considerably in amount as pregnancy advances, so that during the latter half of gestation the total amount of fats and lipoids demonstrable under the microscope becomes quite low (Fig. 522C); but in the last weeks neutral fat is deposited in very large but sparse globules in some of the lutein cells.

By the use of these observations the author (Corner, 1915) is able to distinguish seven stages of pregnancy as reflected in the cells of the corpus luteum of the sow. The corpora lutea of ovulation degenerate (in this species) on the fifteenth day of the cycle, and therefore pass through part of the first stage only.

In the rat Long and Evans find that recently formed lutein cells are laden with small uniform granules of osmium-blackening fat. At the time of the next estrus the number of the granules is increased and their size enlarges. In this species, unlike the pig, each set of corpora lutea persists with little change throughout several succeeding ovulations; but the older corpora may be distinguished from the more recent by the larger number and by the greater and less regular size of the fat granules. The special corpora lutea (so-called corpora lutea of lactation) which are formed by an ovulation immediately after parturition (a constant phenomenon in some rodents) contain lipoid granules which are smaller than those of other corpora lutea.

Van der Stricht (1912) has given a very careful description of the lipoidal inclusions in the corpora lutea of various bats. In these animals there is also a marked accumulation of fatty substances in the first part of the period of gestation, continuing throughout the life of the corpus luteum. As we shall see later, Van der Stricht believes that the lipoidal masses are excreted from the cells into the intercellular spaces and thus into the lymphatic system. Drips (1919) has seen the appearance of lipoidal droplets (in the gopher, *Spermophilus*) during the second half of gestation. It will be seen from these statements that the history of the lipoids of the lutein cells is not quite the same in all species; and indeed Miller (1910) states that the fresh human corpus luteum contains no material which stains with Sudan III or osmium tetroxide, until the degeneration of the corpus luteum is in progress. Mulon (1912) has noted that in early stages (in several species, including man) the corpus luteum contains doubly refractile droplets. From his descriptions, it appears that the cells containing anistropic materials are chiefly or entirely those of the theca interna.

Chemical studies of the fats and lipoids of the ovary have not progressed very far. Chauffard, Laroche and Grigaut (1912) have emphasized the high percentage of cholesterol in the sow's corpus luteum, and Fenger (1914) reports the presence (in the bovine corpus luteum) of about thirteen times

as much phosphorus, soluble in petroleum ether, as in lean muscle. Corner (1917), estimating the alcohol-soluble phosphorus compounds of the sow's corpus luteum, finds the quantity to diminish as pregnancy advances. Further literature will be found in the papers of Hermstein (1925) and Watrin (1924).

We shall recur to this subject in connection with the interstitial cells, but it may be said here, with regard to the whole question of the lipoids of the ovary, that we are still uncertain as to their possible functional rôle. Many writers, especially in France, have been willing to assume that these lipoids represent an active secretion of physiological importance, but experimental proof is still lacking, unless perhaps it is hinted at by the recent work of Allen, Pratt and Doisy (see their paper of 1925 for literature) on hormone-like extracts of the follicle and other genital tissues. It is possible, on the other hand, that the deposits of neutral fats, phosphatides, etc., are merely accompaniments of growth and degenescence in these epithelioid cells. Robertson (1916) has shown that the bodies of young animals contain a much higher percentage of alcohol-soluble phosphorus than those of the old. The corpus luteum of early pregnancy is, in effect, an islet of juvenile tissue among the older cells of its host. Is the larger amount of phospholipins, at this stage, merely an accompaniment of the youthfulness of the organ, or is it connected with the special secretory activities of the cells?

5. Pigment:

As its name indicates, the corpus luteum of some species is characterized by a brilliant yellow or orange pigmentation. The intensity of this color is correlated with the color of the general fat deposits of the body; those animals which are able to store the lipoid-soluble pigments of their foodstuffs store them in the lutein cells as well as in other tissues. No pigment is found in the corpus luteum of the common rodents, of the dog and cat, or of the sheep and pig. On the other hand, the human corpus luteum is bright yellow and that of the cow (to which indeed the adjective *luteum* was first applied by Malpighi) is of a brilliant orange hue. The only microchemical evidence we have as to the nature of this fact is the finding by Escher (1913) that the yellow substance of the cow's lutein tissue is identical with the well-known vegetable pigment, carotin. Palmer and Eckles (1914) have also discovered small quantities of xanthophyll in the cow's corpus luteum, which therefore carries the same pigments as the milk fat.

Histologically the distribution of pigment in the corpus luteum has been little studied. It is commonly stated that the yellow pigment is to be found chiefly in the lutein cell; that is to say, in the large cells forming the bulk of the corpus luteum which we now know to be derived from the granulosa; the commonly applied name of lutein cells is based upon this supposition. However, frozen sections and teased preparations of the fresh human corpus

luteum of early pregnancy show only a little pale yellow pigmentation in the lutein cells themselves; most of the bright color of the tissue is due to the presence of densely packed orange-yellow granulations in a few cells which lie compressed here and there along the vessels and among the large cells. The nature of these pigment-bearing cells is uncertain, and the whole question of the pigmentation of the human corpus luteum needs further investigation, both histologically and chemically.

6. *The question of specific secretory granules in the corpus luteum:*

A number of writers, including especially Cesa-Bianchi (190-) and Drips (1919), have described specific granulations in the lutein cells. Such granules have usually possessed a fuchsinophile reaction after bichromate fixatives, and one is inclined to agree with Levi (1913) that most of the structures described were mitochondria altered by the fixation. O. Van der Stricht (1912) believed that the osmium-stainable lipoid granulations are the specific secretory products of the corpus luteum, and that these granules or their derivatives are actually secreted into tissue spaces and thence into the lymphatic system, to serve as a reproductive hormone. In spite of the undoubted accuracy of his cytological description, this functional hypothesis has not won acceptance; and it is safe to say that up to the present time we have no knowledge of any visible specific secretion of the lutein tissue.

7. *Degeneration of the granulosa lutein cell:*

At the end of the period of functional activity during the ovulation cycle, and after parturition, the granulosa lutein cells disappear from the corpus luteum by degeneration. The process of wholesale destruction which then takes place has been described by Loeb (1911) in the guinea pig; by Togari (1923), in the mouse; and by Corner (1921) in the domestic pig. The lipoid deposits at the periphery of the lutein cells become more extensive until in fixed tissues the vacuoles are so numerous that the nucleus appears to be suspended by a few strands of cytoplasm. Accompanying this fatty degeneration of the cell the nucleus is destroyed either by pycnosis or chromatolysis. The débris thus formed is carried away by polymorphonuclear leucocytes and macrophages.

IV. THECA INTERNA

The cells of the theca interna are not of great cytological or physiological interest during the resting phases of the follicle, but during a brief period preceding maturation and during the subsequent changes which result either in formation of the corpus luteum or in follicular atresia, these cells undergo important changes and no doubt take part in the physiological reactions which occur at this time.

Space is lacking for a discussion of the much controverted question as to the derivation of the follicular layers. Whatever the origin of the theca interna, its cells do not differ in appearance from the fibroblastic type during the period of growth of the follicle (Fig. 523A). At some time during this period they acquire (at least in some species) a few granules of neutral fat. Solomons and Gatenby (1924) state that these cells are stained more or less specifically with Da Fano's silver method. The change which the theca cells undergo during the maturation of the follicle may be described as they are seen in the ovary of the sow, which has been studied extensively in the author's laboratory and in which the chronological relations are fairly well known (Corner, 1919; Seckinger, 1923). It is to be remembered that this

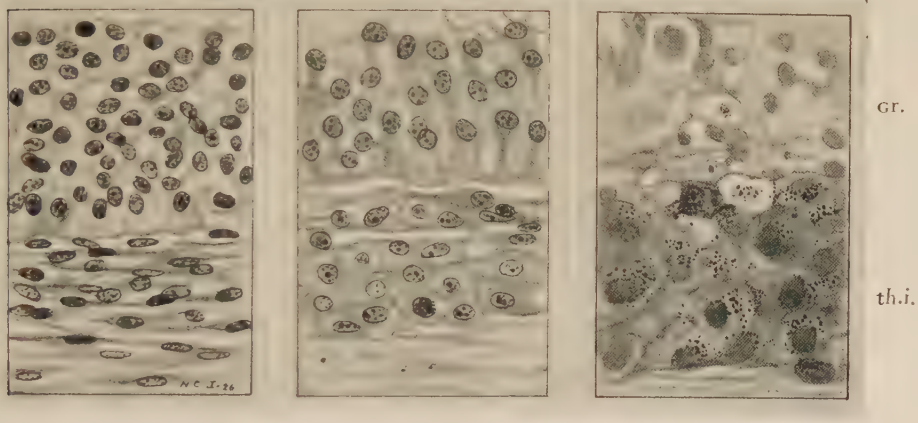


FIG. 523.—Wall of normal follicle, sow. × 500. A, follicle of 7 mm. diameter, showing theca interna cells of fibroblastic type. gr., granulosa; th. i., theca interna. B, follicle of 9 mm. diameter, containing mature ovum, during estrus, showing theca interna cells of epithelioid type. C, the same as B, fixed and stained with osmium tetroxide, showing lipoid inclusions.

animal is one of those which ovulate spontaneously. Those follicles which are to rupture grow suddenly from the resting stage (diameter 5 mm.) to the stage of maturity (diameter 8 to 10 mm.) within a space of three days before the moment of ovulation. During this period the cells of the theca interna divide freely and undergo marked increase in size, reaching diameters of 15μ to 20μ. There is a slight enlargement and vesiculation of the nucleus, together with a great increase in the cytoplasmic inclusions of the cell (Fig. 523B). A large number of osmium-staining fat granules are deposited in most of the cells, together with other granulations which are dissolved during the process of fixation and which therefore leave behind them small, densely packed vacuoles (Fig. 523C). The result of these changes, together with the fairly rich circulation which develops in the inner zone of the theca interna during estrus, is to convert the theca into a tissue with the charac-

teristic structure of the endocrine glands, having cells now of epithelioid type laden with lipoidal and other granulations and richly vascularized.

Similar changes have long been known to occur in other species; Robinson (1918) has described them in the ferret's ovary. Since they appear to be characteristic of the period of maturity of the follicle, it is to be hoped that the exact time of their occurrence in the ovulation cycle will be worked out in other forms. After rupture of the follicle the cells of the theca interna play a part in the formation of the corpus luteum which has been the subject of much disagreement and which is still far from settled, possibly owing to actual differences between the various mammalian species. Following Sobotta (1896) some workers, dealing with the small rodents and with sheep (Marshall, 1903), believe that the theca interna cells are converted into ordinary fibroblasts and accompany the blood vessels as they wander among the granulosa in order to build the connective-tissue framework of the corpus luteum. The author (1919) has described the theca interna cells of the sow as retaining their mature characteristics during the stage of vascular invasion in the formation of the corpus luteum, becoming disseminated throughout the corpus and ultimately losing much or all of their lipoid inclusions. Thus in the author's view and in the one species which he has studied in detail, the theca cells persist even until the last stages of the corpus luteum, forming a second type of epithelioid cell in this endocrine gland. When the granulosa lutein cells break down and disappear, the theca cells, now heavily laden once more with lipoids, persist in the scar (corpus albicans) for many weeks (Corner, 1921). Solomons and Gatenby (1924) have confirmed this description, with a trifling difference as to the degree of dissemination of the theca cells.

In some other mammals and especially in man (Meyer, 1911) the cells of the theca interna are said to linger in their original position, during the formation of the corpus luteum, forming small groups or even a complete zone three or four cells deep about the periphery of the corpus luteum, where they are recognizable by smaller size than the granulosa lutein cells. They are said by Meyer to diminish in numbers and finally to disappear. The author (Corner, 1919) has noted an interesting property of the theca interna cells; namely, that when during the process of rupture of the follicle small haematomata are produced in the theca, its cells can accumulate fairly large masses of blood pigment, by a process morphologically resembling the accumulations of carbon or trypan blue in macrophages.

In the process of follicular atresia the cells of the theca interna also play an important part. As seen in the very simple form of atresia characteristic of the sow, the theca cells proliferate after the disintegration of the granulosa, losing their epithelioid character and reverting to fibroblastic type. It seems probable that they actually contribute to the formation of the dense collagenic fibers which form the bulk of the corpus atreticum, among

which they remain for a long period laden with osmium-blackening inclusions and with a slight amount of yellowish pigment. In the more complicated form of atresia, seen, for example, in the cat as described by Kingsbury (1914), the theca interna cells form distinct clumps or zones of epithelioid cells at the site of the disappearing follicle, which persist so long that their connection with a follicle is obscured, by which time the theca internal cells are in fact "interstitial cells." In the rabbit a similar process continues to such an extent that the stroma of the normal ovary is entirely filled with such masses of interstitial epithelioid cells.

V. THE INTERSTITIAL CELL

In discussing the interstitial cell of the mammalian ovary, it is first necessary to define the term, which is at present subject to much confusion, owing in part to the remarkable histological differences between the ovaries of various species and in part also to differences of opinion as to the classification and significance of the elements of the ovary. The point is best illustrated by a consideration of typical ovaries representing the extremes of structure. The rabbit's ovary, for instance, displays a stroma which consists so largely of epithelioid cells heavily laden with lipoid granules, that the entire organ is in effect a solid mass of these cells in which the follicles and corpora lutea are embedded. This arrangement has led, indeed, to the conception, first put forward by Bouin, that the ovarian stroma in this and similar species is a gland of internal secretion, the so-called interstitial gland. Embryological study shows that in large part these interstitial cells are derived from the theca interna of atretic follicles. This point is not easy to demonstrate when interstitial cells are as numerous as they are in the rabbit, but it is perfectly clear in the cat (Kingsbury, 1914) and some other animals. However, interstitial cells are also found at a very early stage of embryonic differentiation in many species, and here they seem to be produced by the modification of the cells of the stroma and of the various epithelial proliferations. It has even been stated (Rasmussen, 1918) that there is a third source of interstitial cells (in the woodchuck, *Marmota monax);* namely, in cells which proliferate from the germinal epithelium during adult life.

On the other hand, the ovary of the adult domestic pig never contains any epithelioid cells outside of recognizable follicles and corpora lutea; the stroma cells are simply fibroblasts. It has been stated (Allen, 1904) that a few interstitial cells are found in the embryonic ovary, but a recent investigator of the development of the pig's ovary (Wilson, 1924) informs me that he cannot confirm this statement.

Such an ovary as that of the cat is intermediate between the extremes represented by the rabbit and the pig. Here, as already mentioned, inter-

stitial cells are produced in the theca interna of atretic follicles; when in late stages of atresia the outlines of the follicles are obscured, the theca cells remain in more or less discrete clumps at the site of the follicles, whereas in the rabbit they coalesce into a continuous tissue. To add to the confusion it has recently been shown by Velloso de Pinho, as we have already mentioned in another connection, that in the dormouse the granulosa cells persist in atretic follicles and in their turn are converted into interstitial cells indistinguishable from those formed in the theca interna.

In the adult human ovary there appear to be no epithelioid cells outside of recognizable follicles and corpora lutea. Discordant statements on this subject which occur in the literature arise chiefly from the fact that some writers choose to call the theca cells of atretic follicles "interstitial cells" while others do not. It is, of course, quite possible that these cells, which are so much alike, whether found in great numbers in the stroma, as in the rabbit, or in thin layers in atretic follicles, are in fact functionally the same; but proof of this is lacking, and therefore it seems best to avoid confusion by limiting the term interstitial cell to epithelioid elements of the ovarian stroma, outside of recognizable follicles and corpora lutea.

With this somewhat lengthy introductory definition we may discuss the cytology of the interstitial cells. Almost all of the observations in the literature have been made upon the ovaries of the rabbit, the guinea pig, several species of the bat and the domestic cat. The cells are usually polyhedral and of fairly large size, reaching a diameter of from 25μ to 30μ in many species. The nuclei are fairly large and vesicular, and often show considerable variation in intensity of staining. The presence of a Golgi net has been described by Cattaneo (1914) and by Hortega (1913). Mitochondria have been described by Levi (1913) and by Athias (1920), the latter of whom reports that as the interstitial cells enlarge and become laden with fat, the mitochondria become less abundant. He believes that the mitochondria are actually transformed into fat; but similar statements have been made and contradicted with regard to so many other tissues that we may consider this matter in doubt.

In all species so far studied the interstitial cells contain granules of neutral fat or at least of lipoids which reduce osmic acid and stain with Sudan III. Athias finds that these granules are not completely soluble in xylol or balsam (contrary to previous reports), but that they are completely soluble in turpentine. Mulon (1912) has observed anisotropism of the granules, a fact which perhaps suggests the presence of cholesterol. He has also described (1910) the presence of lipoids other than neutral fats, which form a lake with Weigert's hematoxylin. Here again, as in the theca interna of normal follicles and in the corpus luteum, we have the same problem as to the lipoid inclusions; that is to say, whether there is any evidence as to their function. Certain writers have been willing to assume that the lipoids

of the interstitial cell represent a true internal secretion. Van der Stricht (1912) considers that the fatty substances of interstitial cells as well as of the corpus luteum are excreted into the lymphatic system. It is safe to say, however, that current cytological methods teach us nothing about these lipoids except their presence.

Rasmussen's studies on the woodchuck have revealed a marked seasonal variation in the number of interstitial cells and in the amount of lipoid present in them.

VI. EPITHELIUM OF THE FALLOPIAN TUBES

The Fallopian tubes are lined by a single-layered epithelium, consisting of ciliated and non-ciliated cells. Smaller rounded cells of unknown provenience, with deeply staining nuclei and scant cytoplasm, are scattered here and there in the epithelium, close to the basement membrane. Interest in the tubal epithelium has been greatly increased recently by the discovery that its cells participate in the cyclic alterations which take place in the whole genital canal in connection with the ovarian cycle. An excellent review of this subject will be found in the monograph of R. Courrier, 1924. It may be said that all the workers agree as to the existence of cyclic changes, but at present there are two viewpoints as to the rôle of the ciliated and the non-ciliated cells. Courrier and the French workers in general, making use of fixed and stained preparations from several species, believe that the two cell types represent stages of the cycle; that is to say, that one and the same cell periodically sheds and regains its cilia. Moreaux (1913) thus discovers in the rabbit, and Courrier and Gerlinger (1923) in the dog, stages at which the tubal epithelium is not ciliated. Snyder (1923) has studied large numbers of tubes of the sow, always observing the living beating cilia, and checking the fresh tissue by section. In his preparations the cilia are seen to be active at all stages of the cycle, and the number of ciliated cells does not seem to vary. Unpublished observations made by Snyder as occasion has offered, on fresh human tubes from the operating room and on other species, including a few specimens from *Macacus rhesus* at various stages of the menstrual cycle, have invariably shown active cilia. It is to be hoped that this important discrepancy may be checked by further application of the physiological method of observation to other of the species to which the Strasbourg workers have brought their superb staining technique.

The cyclic changes seen by Snyder in the sow may be described as follows: While the ovum is on its way through the oviduct, the epithelial cells of both types are at their maximum height (25μ) and the free surface of the non-ciliated cells is smooth. Later, in the interval between ovulations, the epithelium becomes much lower (10μ) and the non-ciliated cells are beset, upon their free surfaces, with knob-like or finger-shaped cytoplasmic

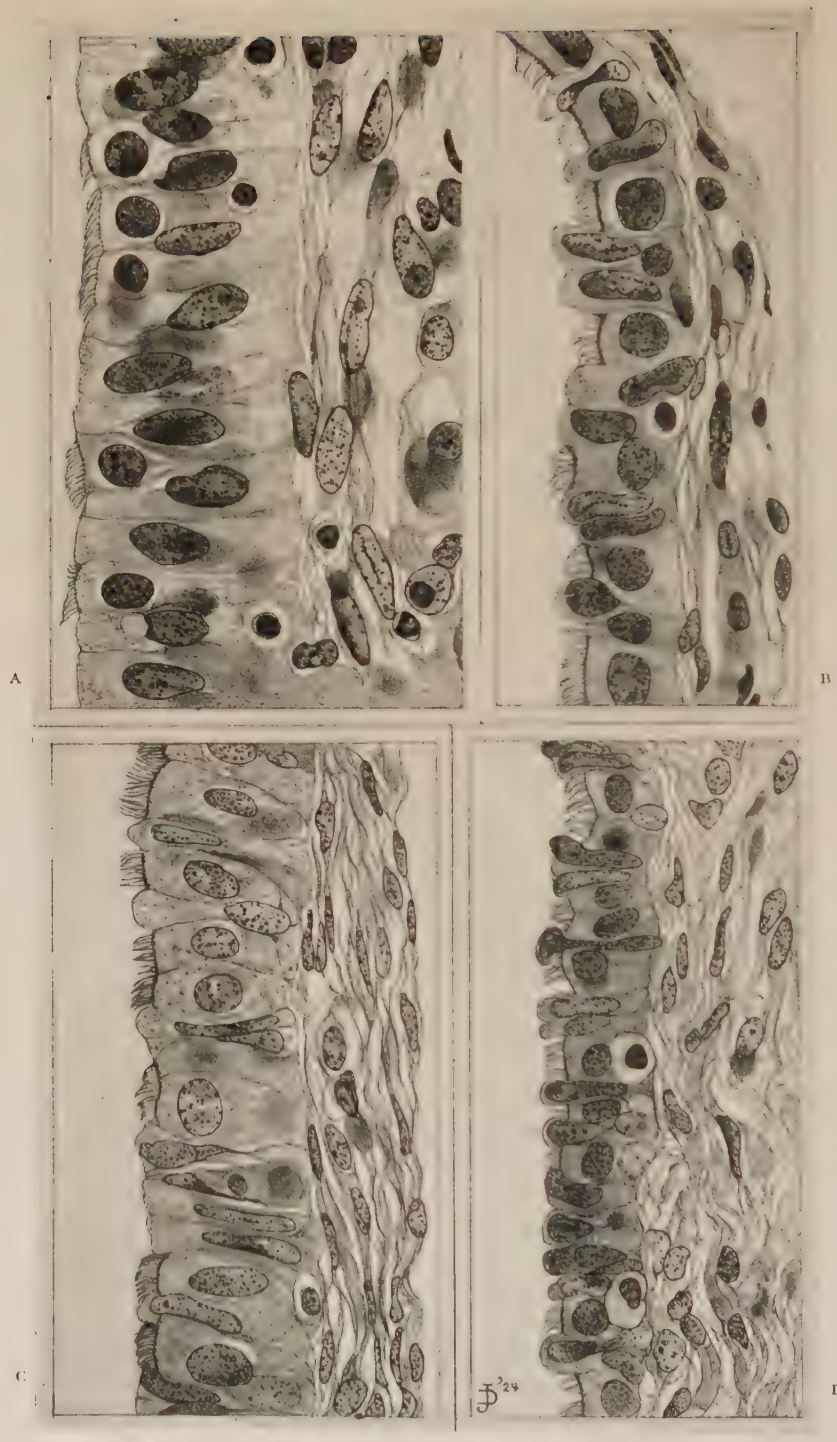


FIG. 524.—Epithelium of human Fallopian tube, showing physiological changes. × 700. (After Snyder, 1924.) A, mid-interval; B, pre-menstrual; C, late interval; D, pregnancy.

processes. Here and there certain of the non-ciliated cells are seen to be so compressed that their nuclei are forced out into the processes, presumably to be cast off into the lumen.

Snyder (1924) has also described almost exactly similar cyclic changes in a series of fixed human tubes (Fig. 524), thus incidentally obtaining an important piece of collateral evidence as to the correlation between the cycles of the pig and the human species.

Spaeth (1923) whose paper appeared shortly before that of Snyder, also sees the very high epithelium at the time of estrus, and, moreover, he describes secretion granules (though not well defined) in the apical portion of the cells. Later in the cycle the same cells, according to this author, become much lower, discharge their secretion granules and regain their cilia.

Courrier and Gerlinger show that in the dog the tubal epithelial cells during estrus are of the usual two types. Some of the ciliated cells do not display basal granules. The non-ciliated cells contain short mitochondrial rods and numerous secretion granules, some of which are fuchsinophile. After estrus, the cells become much lower and regain their cilia. There is no further sign of secretion, but many of the cells undergo the same compression and even enucleation, as described above. We may conjecture that these changes serve in some way to nourish the ovum, or what is more likely, to facilitate its passage through the tube. It has been noticed (Geist, 1913) that in senility the human tubal epithelial cells lose their cilia and accumulate near their free ends numerous fine granules of unknown nature.

VII. BIBLIOGRAPHY

I. OVUM

Asami, G. 1920. Observations on the follicular atresia in the rabbit ovary. *Anat. Record,* **18,** 323.

Athias, M. 1910. Sôbre as divisões de maturacão do óvulo dos mammiferos. *Trab. d. lab. di inst. bacter. Camaro Pestana,* Lisbon. 192 pp.

—— 1915. Cristalloïdes dans l'oeuf de Cercopithecus callitrichus et de Cercopithecus sabaeus. *Bull. de la Soc. Portugaise des Sci. Nat.,* **7,** 67.

Bloor, W. R. 1922. Fat transport in the animal body. *Physiol. Rev.,* **2,** 92.

Branca, A. 1926. L'ovocyte atrésique et son involution. *Arch. de biol.,* **35,** 325.

Charlton, H. H. 1917. The fate of the unfertilized egg in the white mouse. *Biol. Bull.,* **33,** 321.

Clark, E. B. 1923. Observations on the ova and ovaries of the guinea-pig, Cavia cobaya. *Anat. Record,* **25,** 313.

Corner, G. W. 1917. Maturation of the ovum in swine. *Anat. Record,* **13,** 109.

—— 1923. Ovulation and menstruation in Macacus rhesus. *Contrib. Embryol. (Carnegie Inst.), Wash.,* **15** (75) 75.

Corner, G. W., and Amsbaugh, A. E. 1917. Oestrus and ovulation in swine. *Anat. Record,* **12,** 287.

Evans, H. M. 1916. On the behavior of the mammalian ovary and especially of the atretic follicle towards vital stains of the acid azo group. *Proc. Soc. Exper. Biol. Med.*, **13**, 80.

Fischer, A. 1905. Zur Kenntnis der Struktur des Ölemmas der Säugetiereizellen. *Anat. Hefte*, **29**, 555.

Gatenby, J. B. 1922. Some notes on the gametogenesis of *Ornithorhynchus paradoxus*. *Quart. J. Micr. Sc.*, n. s., **66**, 475.

Gatenby, J. B., and Hill, J. P. 1924. On an ovum of Ornithorhynchus exhibiting polar bodies and polyspermy. *Quart. J. Micr. Sc.*, n. s., **68**, pt. 2, 229.

Hartman, C. G. 1916. Studies in the development of the opossum Didelphys virginiana L. *J. Morphol.*, **27**, 1.

Heape, W. 1886. The development of the mole (Talpa europea), the ovarian ovum, and segmentation of the ovum. *Quart. J. Micr. Sc.*, n. s., **26**, 157.

Hill, J. P. 1910. The early development of the Marsupialia, with special reference to the Native Cat (Dasyurus viverrinus). *Ibid.*, **56**, 1.

Hirschler, J. 1917. Über die Plasmakomponenten (Golgischer Apparat, Mitochondrien u. a.) der weiblichen Geschlechtzellen (zytologische Untersuchungen am Ascidien-ovarium). *Arch. f. mikr. Anat.*, **89** (Abt. 2), 1.

Hubrecht, A. A. W. 1902. Furchung and Keimblattbildung bei Tarsius spectrum. *Verh. k. akad. Wetenschappen*, Amsterdam, Sect. 2, **8**, 1.

Kulesch, L. 1914. Der Netzapparat von Golgi in den Zellen des Eierstockes. *Arch. f. mikr. Anat.*, **84**, 142.

Lams, H. 1913. Étude de l'oeuf de cobaye aux premiers stades de l'embryogenèse. *Arch. de biol.*, **28**, 229.

Lams, H., and Doorme, J. 1907. Nouvelles recherches sur la maturation et la fécondation de l'oeuf des mammifères. *Ibid.*, **23**, 259.

Levi, G. 1915. Il comportamento dei condriosomi durante i piu precoci periodi dello sviluppo dei Mammiferi. *Arch. f. Zellforsch.*, **13**, 471.

Loeb, L. 1911. The parthenogenetic development of ova in the mammalian ovary and the origin of ovarian teratomata and chorio-epitheliomata. *J. Am. Med. Assoc.*, **56**, 1327.

Long, J. A., and Mark, E. L. 1911. The maturation of the egg of the mouse. *Carnegie Inst. Wash., Pub. 142.*

Longley, W. H. 1911. The maturation of the egg and ovulation in the domestic cat. *Am. J. Anat.*, **12**, 139.

Mertens, H. 1895. Recherches sur la signification du corps vitellin de Balbiani dans l'ovule des mammifères et des oiseaux. *Arch. de biol.*, **13**, 390.

Monterosso, B. 1915. Su l'origine e la costituzione dei materiali deutoplasmici nell' oocite in accrescimento dei Mammiferi. *Arch. f. Zellforsch.*, **13**, 530.

Moore, J. E. S., and Tozer, F. 1908. On the maturation of the ovum in the guinea-pig. *Proc. Roy. Soc. Ser. B.*, **80**, 285.

Nagel, W. 1888. Das menschliche Ei. *Arch. f. mikr. Anat.*, **31**, 342.

Nihoul, J. 1926. Recherches sur l'appareil endocellulaire de Golgi dans les premières stades de développement des mammifères. *La Cellule*, **37**, 23.

Rubaschkin, W. 1905. Ueber die Reifungs- und Befruchtungsprozesse des Meerschweinchencies. *Anat. Hefte*, **29** (Abt. 1), 507.

——— 1907. Ueber die Veränderungen der Eier in den zugrundegehenden Graafschen Follikeln. *Ibid.*, **32** (Abt. 1), 255.

Russo, A. 1910. Sui mutamenti che subiscono i mitochondri ed i materiali deutoplasmici dell' oocite di coniglia in diversi periodi di inanizione. *Arch. f. Zellforsch.*, **5**, 173.

—— — 1912. Aumento dei granuli protoplasmici nell' oocite delle coniglie inietatte con lecitina, loro diminuzione nelle coniglie digiunanti, e loro natura lipoide e mitochondriale. *Ibid.*, **8**, 203.

Sjövall, E. 1906. Ein Versuch, das Binnennetz von Golgi-Kopsch bei der Spermato- und Ovogenese zu homologisieren. *Anat. Anz.*, **28**, 561.

Sobotta, J., and Burckhard, G. 1910. Reifung und Befruchtung des Eies der weissen Ratte. *Anat. Hefte*, **42**, 433.

Stockard, C. R., and Papanicolaou, G. N. 1917. The existence of a typical oestrous cycle in the guinea-pig with a study of its histological and physiological changes. *Am. J. Anat.*, **22**, 225.

Thing, A. 1918. The formation and structure of the zona pellucida in the ovarian eggs of turtles. *Am. J. Anat.*, **23**, 237.

Thomson, A. 1919. The maturation of the human ovum. *J. Anat.*, **53**, 172.

Tur, J. 1912. Sur l'origine de la zone pellucide des oeufs des mammifères. *Compt. rend. Soc. d. biol.*, **72**, 336.

Van Beneden, E., and Julin, C. 1880. Observations sur la maturation, la fécondation, et la segmentation de l'oeuf chez les cheiroptères. *Arch. de biol.*, **1**, 551.

Van der Stricht, O. 1908. La structure de l'oeuf de la chienne et la genèse du corps jaune. *Compt. rend. Soc. des Anat.*, 10e Reunion, Marseilles, 1.

1909. La structure de l'oeuf des mammifères (Chauve souris, Vesperugo noctula), troisième partie. *Mém. Acad. roy. Belgique*, Bruxelles, 2e Ser., **2**, 1.

—— 1923. Étude comparée des ovules des mammifères aux différentes périodes de l'ovogenèse, d'après les travaux de Laboratoire d'Histologie et d'Embryologie de l'Université de Gand. *Arch. de biol.*, **33**, 229.

Van der Stricht, R. 1911. Vitellogenèse dans l'ovule de chatte. *Arch. de biol.*, **26**, 365.

Weigl, R. 1912. Vergleichend-zytologische Untersuchungen über den Golgi-Kopschen Apparat und dessen Verhältnis zu anderen Strukturen in den somatischen Zellen und Geschlechtszellen verschiedene Tiere. *Bull. Acad. Sci. Cracow*, 1912B, 417.

Wilson, J. T., and Hill, J. P. 1908. Observations on the development of Ornithorhynchus. *Phil. Trans. Roy. Soc.*, **199**, B, 31.

II. THE OVARY (GRANULOSA, LUTEIN CELL, THECA INTERNA, INTERSTITIAL CELL)

Aimé, P. 1906. Les cellules interstitielles de l'ovaire chez le cheval. *Compt. rend. Soc. d. biol.*, **61**, 250.

—— 1907. Recherches sur les cellules de l'ovaire chez quelques mammifères. *Arch. zool. expér. et gén.* (Ser. 4), **7**, 95.

Allen, B. M. 1904. The embryonic development of the ovary and testis of the mammals. *Am. J. Anat.*, **3**, 89.

Allen, E., Pratt, J. P., and Doisy, E. A. 1925. The ovarian follicular hormone. *J. Am. Med. Assoc.*, **85**, 399.

Athias, M. 1911. Observations cytologiques sur l'ovaire des mammifères. 1. Les cellules interstitielles de l'ovaire chez le cobaye (foetus à terme et nouveau-né). *Anat. Anz.*, **39**, 238.

—— 1920. Recherches sur les cellules interstitielles de l'ovaire des cheiroptères. *Arch. de biol.*, **30**, 89.

Benckiser, A. 1884. Zur Entwickelungsgeschichte des Corpus luteum. *Arch. f. Gynäk.*, **23**, 350.

Benthin, W. 1911. Ueber Follikelatresie in Säugetierovarien. *Arch. f. Gynäk.*, **94**, 599.

Borell, H. 1919. Untersuchungen über die Bildung des Corpus luteum und der Follikelatresie bei Tieren, mit Hilfe der vitalen Farbung. *Beit. z. pathol. Anat.*, **65**, 108.

Cattaneo, D. 1914. Richerche sulla struttura dell' ovario dei mammiferi. *Arch. Ital. di Anat. e di Embriol.*, **12**, 1.

Cesa-Bianchi, D. 1906 a. Osservationi sulla struttura e sulla funzione della cosi della ghiandola interstitiale dell' ovaio. *Arch. di fisiol.*, **4**, 523.

——— 1908. Di alcune particolarita di struttura e dei fenomeni di secrezione del corpo luteo. *Internat. Monatsch. f. Anat. u. Physiol.*, **25**, 1.

Chauffard, A., Laroche, G., and Grigaut, A. 1912. Fonction cholesterogénique du corps jaune. Preuves histologiques et chimiques. *Compt. rend. Soc. d. biol.*, **72**, 223, 265.

Cohn, F. 1903. Zur Histologie und Histogenese des Corpus luteum und des interstitiellen Ovarialgewebes. *Arch. f. mikr. Anat.*, **62**, 745.

Corner, G. W. 1915. The corpus luteum of pregnancy, as it is in swine. *Contrib. Embryol. (Carnegie Inst.), Wash.*, **2** (5), 71.

——— 1917. Variation in the amount of phosphatides in the corpus luteum of the sow during pregnancy. *Jour. Biol. Chem.*, **29**, 141.

——— 1919. On the origin of the corpus lutuem of the sow from both granulosa and theca interna. *Am. J. Anat.*, **26**, 117.

——— 1921. Cyclic changes in the ovaries and uterus of the sow, and their relation to the mechanism of implantation. *Contrib. Embryol. (Carnegie Inst.), Wash.*, **13**, (64). 117.

Drips, D. 1919. Studies on the ovary of the spermophile with special reference to the corpus luteum. *Am. J. Anat.*, **25**, 117.

Escher, H. H. 1913. Ueber den Farbstoff des Corpus luteum. *Ztschr. f. physiol. Chem.*, **83**, 198.

Evans, H. M. 1916. On the behavior of the mammalian ovary and especially of the atretic follicle towards vital stains of the acid azo group. *Proc. Soc. Exper. Biol. Med.*, **13**, 80.

Fenger, F. 1914. Distinction between the corpus luteum of ovulation and the true corpus luteum of pregnancy. Preliminary report. *J. Am. Med. Assoc.*, **62**, 1249.

Hermstein. 1925. Untersuchungen über den Lipoidgehalt des Corpus luteum. *Arch. f. Gynäk.*, **124**, 739.

Honoré, C. 1900. Recherches sur l'ovaire du lapin. *Arch. de biol.*, **16**, 537.

Hortega, P., del Rio. 1913. Details nouveaux sur la structure de l'ovaire. *Trab. del Laborat. de Investig. biol. de la Univ. de Madrid*, **11**, 163.

Kingsbury, B. F. 1914. The interstitial cells of the mammalian ovary: Felis domestica. *Am. J. Anat.*, **16**, 59.

Levi, G. 1913. Note citologiche sulle cellule somatiche dell' ovaio dei Mammiferi. *Arch. f. Zellforsch.*, **11**, 515.

Limon, M. 1903. Étude histologique et histogénique sur la glande interstitielle de l'ovaire. *Arch. d'anat. micr.*, **5**, 155.

Loeb, L. 1911. The cyclic changes in the ovary of the guinea-pig. *J. Morphol.*, **22**, 37.

Long, J. A., and Evans, H. M. 1922. The oestrous cycle in the rat and its associated phenomena. *Mem. Univ. California*, **6**.

Marshall, F. H. A. 1903. The oestrous cycle and the formation of the corpus luteum in the sheep. *Phil. Trans. Roy. Soc.*, **196**,B, 47.

Meyer, R. 1911. Ueber Corpus luteum-Bildung beim Menschen. *Arch. f. Gynäk.*, **93**, 354.

——— 1911. Zur Corpus luteum-Bildung beim Menschen. *Zentralbl. f. Gynäk.*, **35**, 1206.

Miller, J. W. 1910. Die Rückbildung des Corpus luteum. *Arch. f. Gynäk.*, **91**, 263.

——— 1911. Ueber Corpus-luteum-Bildung beim Menschen. Kritische Bemerkungungen zu der im Archiv für Gynäkologie Bd. XCIII, Heft 2, erschienenen Arbeit Prof. Dr. Meyer's. *Zentralbl. f. Gynäk.*, **35**, 1089.

Mulon, P. 1910. Sur une sécrétion lipoïde nouvelle de la glande interstitielle ovarienne. *Compt. rend. Soc. d. biol.*, **68**, 423.

— — 1912. Les corps birefrangents des glandes genitales. *Ibid.*, **72**, 204.

Palmer, L. S., and Eckles, C. H. 1914. Carotin, the principal natural yellow pigment of milk fat. Part III. *Research Bull. No. 11, Missouri Agr. Exp. Sta.*, 391.

Pardi, U. 1915. Sur les cellules interstitielles ovariques de la lapine et sur les elements de la thèque interne de l'ovaire humaine hors de la gestation et durant celle-ci. *Arch. Ital. d. biol.*, **62**, 353.

Parhon, C., Dumitresco, G., and Nissipesco, C. 1909. Note sur les lipoïdes des ovaires. *Compt. rend. Soc. d. biol.*, **66**, 650.

Rasmussen, A. T. 1918. Cyclic changes in the interstitial cells of the ovary and testis in the woodchuck (Marmota monax). *Endocrinology*, **2**, 353.

Riquier, S. K. 1910. Der innere Netzapparat in den Zellen des Corpus luteum. *Arch. f. mikr. Anat.*, **75**, 772.

Robertson, T. B. 1916. Experimental studies on growth. II. The normal growth of the white mouse. *J. Biol. Chem.*, **24**, 363.

Robinson, A. 1918. Ovarian follicles in ferret and ferret-polecat hybrids. *Trans. Roy. Soc. Edinburgh*, **52**, 302.

Salazar, A. L. 1919. Sur la période chromatolytique de la granulosa atrésique de la lapine. *Mém. Soc. Portugaise des Sci. Nat., Ser. Biol.* **No. 2**, 1.

1922. Formes leucocytoides des corpuscles de la chromatolyse atrésique ayant donné origine à des erreurs d'interprétation. *Bull. Soc. Portugaise des Sci. Nat.*, **9**, 21.

1923. On the existence of special cells with tannophil bodies in the interstitial gland of female rabbit. *Anat. Record*, **26**, 57.

— 1924*a*. Sur l'existence de granulations paranucléaires dans les cellules luteiniques de la lapine, colorables électivement par le tannin-fer. *Compt. rend. Soc. d. biol.*, **91**, 221.

— 1924*b*. En quoi consiste l'atypie des cineses de la granulosa ovarienne et pourquoi cette atypie doit-être considerée comme le prélude de l'atrésie. *Ibid.*, **90**, 586.

Seckinger, D. L. 1923. Spontaneous contractions of the Fallopian tube of the domestic pig with reference to the oestrous cycle. *Johns Hopkins Hosp. Bull.*, **34**, 236.

Sobotta, J. 1896. Ueber die Bildung des Corpus luteum bei der Maus. *Arch. f. mikr. Anat.*, **47**, 261.

Solomons, B., and Gatenby, J. W. B. 1924. Notes on the formation, structure, and physiology of the corpus luteum of man, the pig, and the duck-billed platypus. *J. Obstet. and Gyn. Brit. Emp.*, **31**, 580.

Thomson, A. 1919. The ripe human Graafian follicle together with some suggestions as to its mode of rupture. *J. Anat.*, **54**, 1.

Togari, C. 1923. On the origin of the corpus luteum of the mouse. *Aichi J. Exp. Med.*, **1**, 1.

Van der Stricht, O. 1912. Sur le processus de l'exercétion des glandes endocrines: le corps jaune et la glande interstitielle de l'ovaire. *Arch. de biol.*, **27**, 585.

Velloso de Pinho, A. 1925. Sur une forme particuliére de transformation folliculaire caractéristique de l'ovaire du lérot (Eliomys quercinus, L.): faux corps jaunes métaplastiques. *Anat. Record*, **30**, 211.

Watrin, M. 1924. Étude histochimique et biologique du corps jaune de la femme. *Arch. internat. de méd. exp.*, **1**, 97.

Wilson, K. M. 1924. A morphological study of some phases in the development of the sex gland of the domestic pig. *Am. J. Obstet. and Gyn.*, **8**, 710.

Winiwarter, H. v. 1908. Das interstitielle Gewebe der menschlichen Ovarien. *Anat. Anz.*, **33**, 1.

III. EPITHELIUM OF THE FALLOPIAN TUBE

Courrier, R. 1924. Le cycle sexuel chez la femelle des mammifères. Étude de la phase folliculaire. *Arch. de biol.*, **34**, 369.

Courrier, R., and Gerlinger, H., 1923. Le cycle glandulaire de l'epithélium de l'oviducte chez la chienne. *Compt. rend. Soc. d. biol.*, **87**, 1363.

Geist, S. H. 1913. Die senile Involution der Eileiter. *Arch. f. mikr. Anat.*, **81** (Abt. 1), 220.

Gianelli, L. 1907. Richerche istologiche sull' ovidutto dei mammiferi. *Arch. Ital. anat. e embriol.*, **6**, 1.

Moreaux, R. 1913. Recherches sur la morphologie et la fonction glandulaire de l'epithélium de la trompe uterine chez les mammifères. *Arch. anat. micr.*, **14**, 515.

Schaffer, J. 1908. Ueber Bau und Funktion des Eileiterepithels beim Menschen und bei Säugetieren. *Monatschr. f. Geburts. u. Gynäk.*, **28**, 526, 666.

Snyder, F. F. 1923. Changes in the Fallopian tube during the ovulation cycle and early pregnancy. *Johns Hopkins Hosp. Bull.*, **34**, 121.

——— 1924. Changes in the human oviduct during the menstrual cycle and pregnancy. *Ibid.*, **35**, 141.

Spack, A. 1923. Le cycle oestrien dans l'oviducte de la truie. *Compt. rend. Soc. d. biol.*, **88**, 450.

Tröscher, H. 1917. Ueber den Bau und die Funktion des Tubenepithels beim Menschen. *Monatschr. f. Geburts. u. Gynäk.*, **45**, 205.

Section XXXIII

CELLULAR CHANGES IN THE FLUID OF THE MAMMALIAN VAGINA

CONTENTS

Section XXXIII

PAGE

I. Fluid of Vagina 1154
II. Cellular Movements Accompanying Changes in Nature of Vaginal Fluid 1155
III. Cyclical Changes in Uterus and Vagina Giving Rise to Cell Changes in Vaginal Smear. 1162
IV. Ovary and Cells of Vaginal Smear 1167
V. Nature of Estrous Process. 1169
VI. Bibliography. 1170

Section XXXIII

CELLULAR CHANGES IN THE FLUID OF THE MAMMALIAN VAGINA

CHARLES R. STOCKARD

MANY fluids and excretions of the animal body carry free or loose cells which characterize them in a very typical way. The cells of the blood stream are the best known examples, and the nature and relative abundance of these cells are most significant in indicating important conditions occurring in the individual. The cells in the urine, cerebrospinal fluid, milk and other juices are indicative of the state of the particular organs from which they arise and at times are of even more general importance. Free cells in the fluids of the body are in most cases simply discards or waste products, yet a study of the types and changes in relative numbers of such cells may furnish information as to the actual physiological processes which cause their elimination from the organized tissues. In fact, any change in the nature and number of these cells must indicate the extent of variation in cellular excretion taking place in the region of their source.

The student of normal cytology has given only little attention to cellular waste in analyzing physiological changes occurring in the various organs. Up to 1917 the cells of the vaginal discharge in the mammalian female had hardly been more than casually examined microscopically. There was no idea of their importance as delicate indicators of the changing states in the uterine wall and other parts of the reproductive tract as well as in the ovary itself. Heape (1894, 1897, 1899) had recorded in a general way that the menstrual discharge from the monkey's vagina showed not only red corpuscles but leucocytes and pieces of thrown-off uterine epithelium. No daily examinations were made of the intermenstrual mucus, or slime, and no systematic study of periodic changes in the cellular composition of the smear as related to changes in the uterine wall and ovaries was attempted or even suggested. In investigating the sexual period of the female all workers either killed the animals and studied their genital tract or removed the uterus by operation and examined microscopic sections of its wall. Heape and Van Herwerden (1906) made such studies of the monkey. Several investigators studied the human uterus in a similar way and Leo Loeb (1911, 1914) and others studied with closely similar methods the ovaries and reproductive tract of guinea pigs, rats, dogs and other mammals. These studies failed to furnish a clear understanding of the rhythmic changes in a given living female and none of them made it possible to locate exactly the actual moment of ovulation. However, this is just the knowledge so necessary in an experimental study of the female reproductive processes.

The female reproductive tract undergoes such pronounced structural changes in association with its physiological activities that no effort should be spared in attempting to discover some easily obtainable indicator that will accurately inform us of the varying states of these internal reactions. The marked periodic changes in the genital tract are presumed to be closely

correlated with and actually caused by important and primary processes of growth and differentiation in the cellular organization of the mammalian ovary. After removal or degeneration of the ovaries, it is a well-known fact that all of the rhythmic processes in the genital tract cease. It becomes necessary, therefore, to analyze the cellular nature of the fluids from the genital tract as a possible means of accurately detecting the activities of the ovary, the moment of ovulation and the time of corpora lutea formation. The systematic investigation of the vaginal fluid from this point of view was first recorded from studies on the guinea pig in 1917 (Stockard and Papanicolaou).

The cytological study of the vaginal fluid has made it clearly possible to correlate variations in number and type of the free cells in this fluid with the exact stages of ovarian follicular growth and ovulation. The actual time of ovulation may be as accurately recognized by examining vaginal smears in the mammal as is possible from oviposition in the bird.

Following the analysis of the vaginal discharge in the guinea pig, a number of workers made similar investigations on different mammals (Long and Evans, 1920, 1922, on the rat; Edgar Allen, 1922, the mouse; Corner, 1923, the monkey; Hartman, 1923, the opossum; H. S. Murphey, 1924, the cow; Tsu, 1924, the rabbit, etc.), and all have found a strikingly uniform correlation between the particular cellular composition of the vaginal smear and the several stages in the process of follicular growth and ovulation.

Since the cytology of the smear is so closely similar in a number of mammals we may for convenience base the present description on the conditions as recognized in the guinea pig.

I. FLUID OF THE VAGINA

When the fluid content of the vagina is examined macroscopically in the living guinea pig it is found to change in amount and consistence at regular intervals. A definite sexual period, or estrus, occurs, which lasts for about 24 hours and returns with striking regularity every fifteen to seventeen days. Throughout this 24-hour period fluid is abundant in the vagina and during the first half of the time is of a foamy, mucous consistence. The vaginal fluid then changes to become thick and rather cheesy and finally becomes slowly liquefied and serous. The final thin fluid disappears after a few hours. Rarely toward the end of the process a slight trace of blood may occur, giving a red appearance, but as a rule the fluid is milky-white or cream color.

The changes in appearance may be divided into four different stages: First, the stage of rather clear, frothy mucus lasting from six to twelve hours, or even longer, with gradual increase in quantity until it becomes very abundant and fills the entire lumen of the vagina. The female will

accept the male and copulation occurs only during this first stage when the frothy fluid is present. The second stage, during which the vaginal content presents a cheesy consistence, is shorter, lasting from two to four hours, and passing gradually over into the third thin, serous fluid stage, which may last from five to ten hours. A fourth stage with occasional slight bleeding then follows. Other minor changes are designated by some workers, but for general description these four chief stages are of greatest importance. The succession in which the stages follow one another is remarkably definite.

After every period of sexual activity in the guinea pig an epithelial membrane, termed the "vaginal closure membrane," grows over the opening of the vagina and closes it completely. If this closure membrane be broken and the vagina examined during the resting period, or diestrum, only a scant amount of slimy fluid is present and this contains few cells as compared with the fluid from the four active stages of estrus. The "vaginal closure membrane" has not been found in any other mammal; so far as is known, it is unique for the guinea pig. The presence of this diestral membrane makes easy the recognition of the resting condition in these animals.

II. CELLULAR MOVEMENTS ACCOMPANYING THE CHANGES IN NATURE OF THE VAGINAL FLUID

The cellular compositions of smears prepared from the vaginal fluid at the several stages designated above show decidedly typical differences. The relative proportions of the various cell types in the fluid at different stages are so definite that with little experience one may diagnose the exact sexual state of the animal concerned solely by an examination of the smear.

The mucous fluid of the first stage contains an abundant mass of cells which, as seen in Figures 525 and 527, are of a squamous type and show considerable plasmolysis with bent and wrinkled cell membranes. The nuclei of these cells are very small and pycnotic, sometimes broken into pieces and then appearing as coarse black grains. The cell protoplasm has degenerated, having only a weak affinity for the plasma stains, and exhibits a reticular structure. Such cells derived from the wall of the vagina (Fig. 539) characterize this first stage by their great abundance over other types. There are also to be seen, particularly toward the end of the first stage, a certain number of elongate, cornified cells without nuclei, which are desquamated in larger masses from the more external portions of the vagina. These cells contrast in appearance with the earlier type cells, since in smears stained with hematoxylin and eosin they present a decidedly red color, while the early first type cells are almost gray. The red cells rather serve to indicate an intermediate period between the first and second stages of the flow, and may really be found during both stages but particularly at the end of the first or the beginning of the second stage. In addition to the two types, other

kinds of cells may also be found in a first stage smear but these are never present in abundance nor are they so characteristic as the two just mentioned. Leucocytes are very scarce or strikingly absent from the first-stage smear. All of of the cells float freely in the mucous fluid without assuming any definite arrangement.

During the second stage the vaginal fluid becomes crowded with enormously increasing numbers of cells, thus bringing about the cheese-like consistence of the discharge. These cells, illustrated by Figures 526 and 528,

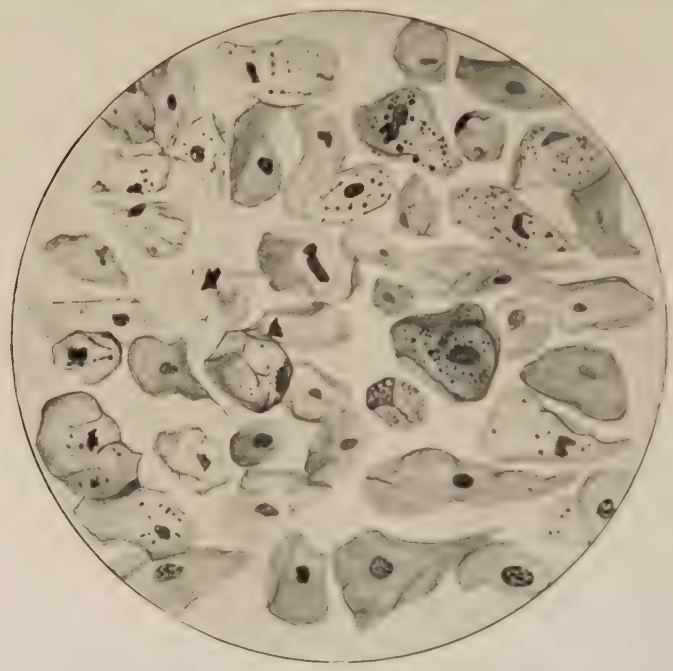


FIG. 525.—Cells typical of the "first stage" of estrus or "heat" in the guinea pig. These are desquamated squamous epithelial cells from the wall of the vagina and are greatly plasmolized and shriveled. The nuclei are small and pycnotic and in some cells there are many coarse chromatin particles scattered throughout. These cells float freely in a clear mucous fluid.

are derived from the upper regions of the vagina with a few from the uterus and they exhibit to a high degree the healthy architecture of an epithelial cell in contrast to the plasmolized first-stage cells. The nuclei are fairly well preserved, showing only slight signs of degeneration. The protoplasm has not greatly deteriorated and gives a good staining reaction, thus differing from the gray-staining first cells. The second stage cells are present in great quantities while the mucous secretion diminishes more and more until it almost disappears. This stage is of short duration—only two to four hours.

In all the mammals that have been studied the breaking of the Graafian follicles and the act of ovulation occurs just as the second stage ends.

The third stage begins with the liquefaction of the cheesy mass. Cells of the second stage become less and less numerous, while myriads of polymorphonuclear leucocytes appear among them. This shower of leucocytes is a most striking occurrence following immediately after ovulation. As the shifting in cellular composition proceeds, finally each of the epithelial cells becomes isolated from others of its kind and lies in the midst of a

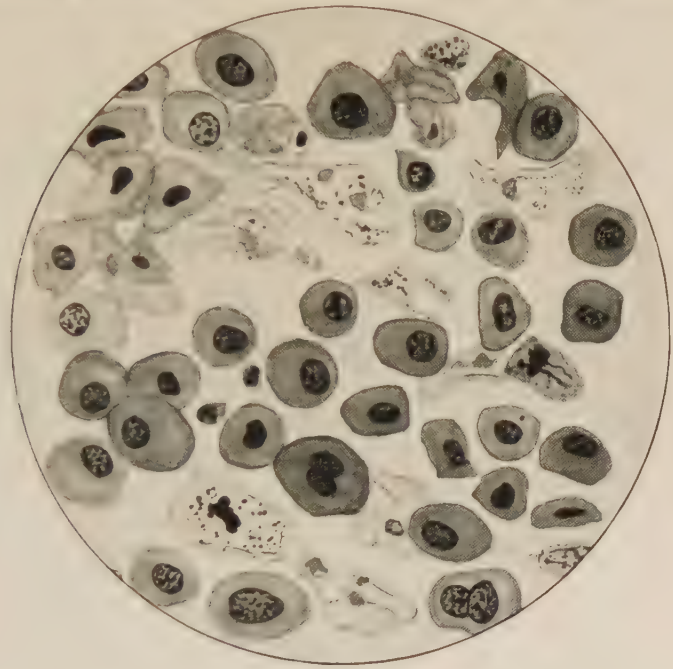


FIG. 526.—Cells of the "second stage" of estrus as seen in a smear from the vaginal fluid of a guinea pig. A few of the first-stage cells of Figure 525 are still present, but the smear is characterized by a great number of fresh, well preserved, strongly staining epithelial cells from the vagina with some from the uterine wall, and an occasional leucocyte may be present. The fluid at this time is no longer foamy mucus but thick and cheesy in consistence.

number of leucocytes (Figs. 529, 531, and 532). The apparent effect of the leucocytes is to dissolve or digest the desquamated epithelial cells, and this dissolving effect is not only noticed in the cells surrounded by leucocytes but in some cases the leucocytes actually dissolve their way into the interior of the cell bodies (Figs. 529 and 531). These appearances are never due, as might possibly be supposed, to the epithelial cells having devoured the leucocytes. The destructive influence of the leucocytes begins before the desquamated epithelial cells have fallen away from the wall of the uterus

and vagina (Figs. 538 and 530), yet it probably continues after the cells are free in the lumen of the vagina. The dissolving power of the leucocytes, which apparently causes the liquefaction of the cheesy mass of epithelial cells, is shown very well when the leucocytes are within a cell and the nucleus is beginning to dissolve. The nucleus is apparently digested and dissolved by coming in contact with the leucocyte without being at all engulfed or enclosed within its smaller body (Fig. 529).

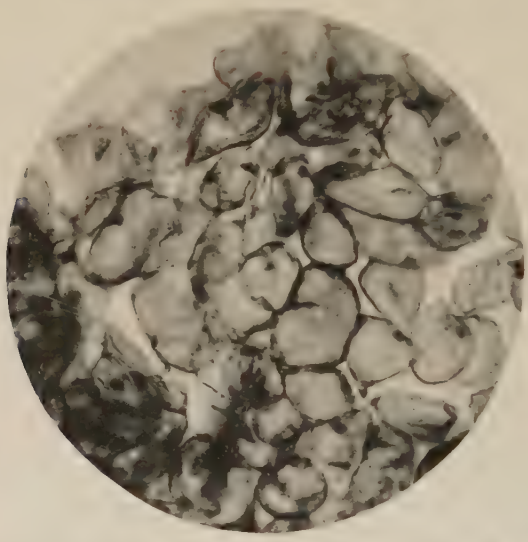


FIG. 527. A photomicrograph of the "first stage" cells showing more exactly their appearance than does the detail drawing, Figure 525.

As the third stage nears its end the material within the vagina is a thin fluid containing a great number of leucocytes as well as many epithelial cells of the second stage type, some of which carry leucocytes within their bodies. These leucocyte-containing cells are fairly typical of the third stage. As would be expected, the leucocytes within the cells very soon show signs of degeneration, never staining so clearly as the free ones outside.

The fourth stage shows the same condition as the preceding but often at this time a slight hemorrhage takes place, although this does not always occur. The hemorrhagic fluid contains in addition to the great number of red blood corpuscles, a large number of leucocytes and also desquamated cells of the second stage, some of which are penetrated by leucocytes (Fig. 530). At times red blood corpuscles become enclosed within the bodies of the leucocytes and are there digested. This may probably be a truly phagocytic action and not entirely the same as the dissolving effect on the neighboring epithelial cells within the fluid.

The presence of leucocytes is not alone confined to the end of the heat period but a number of them is also be to found in the lumen of the vagina during diestrum, Fig. 533. The only time that leucocytes are absent from the vaginal lumen is during the first and second stages of the estrus, as described above. It is interesting that copulation occurs during the height of the first stage when the vagina is filled with the frothy mucous secretion and the leucocytes are entirely absent—both of these conditions contribute

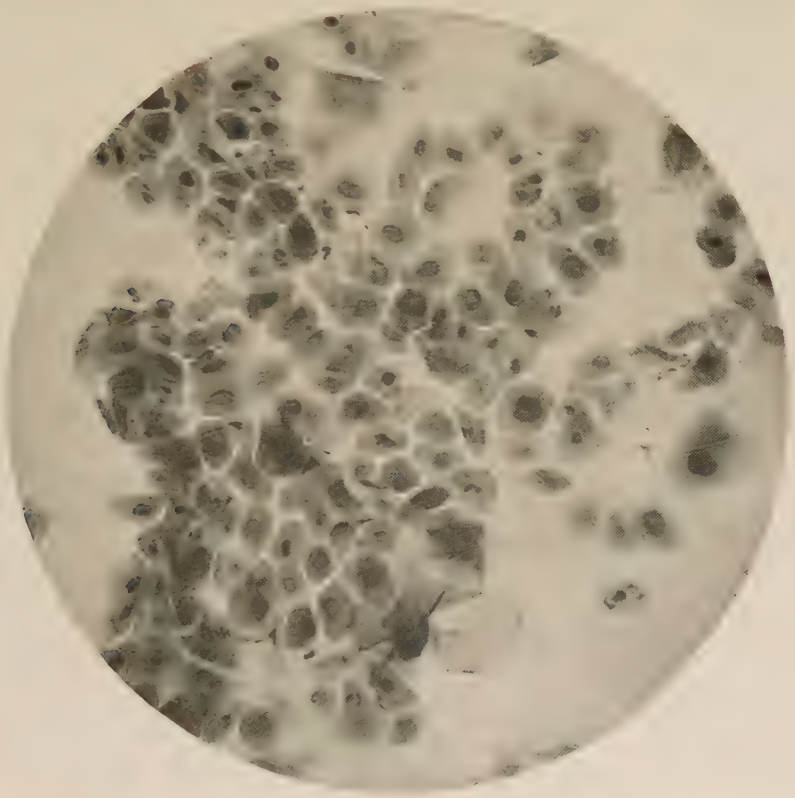


FIG. 528.—Cells of the second state showing the general appearance of the types in Figure 526.

to the success of copulation and fertilization. Throughout the first week after heat the scant fluid which exists in the vagina contains chiefly leucocytes and a few atypical desquamated cells. During the second week the number of epithelial cells increases steadily, and among these atypical cells there may exist isolated cells of the first or of the second stage type.

At the fourteenth and fifteenth days after the beginning of the last estrus first stage cells gradually become more abundant and, the growing

predominance of these cells with the disappearance of leucocytes, indicates the approach of the new period of heat.

The absence of leucocytes from the vaginal lumen at the time of copulation is important, since, if present, they might by their dissolving powers or phagocytic action exert an injurious effect on the spermatozoa and thus interfere with their normal function. Later a special purpose of the leucocyte seems to be to destroy the excess of spermatozoa remaining in the uterus. This frequently occurs by an interesting process of phagocytosis. A leuco-

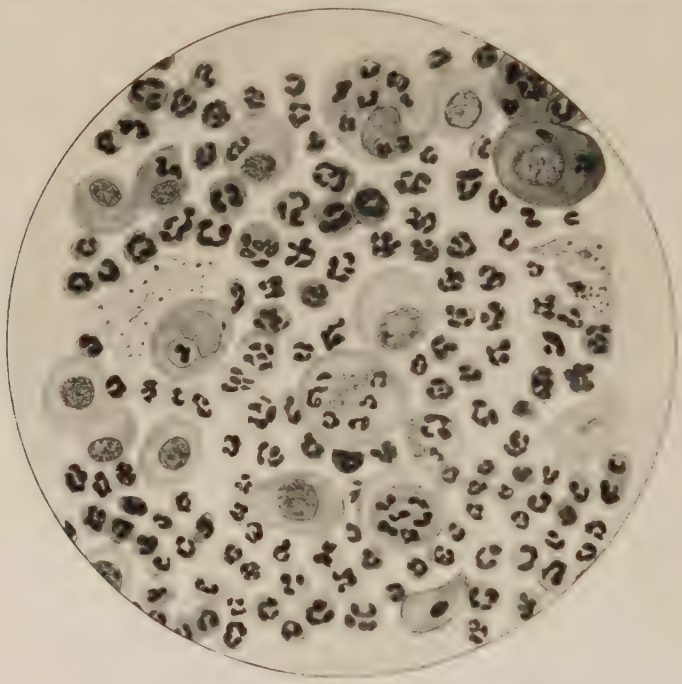


FIG. 529.—A drawing of a smear from the "third stage" fluid. This shows the arrival of myriads of leucocytes into the vaginal fluid; the epithelial cells lie scattered among them and are being dissolved and invaded by the leucocytes. Several cells show many leucocytes within their bodies. The vaginal fluid at this stage has become a thin serous liquid.

cyte comes in contact with a spermatozoon which with its tail is longer than the leucocyte. The leucocyte by stretching and contracting finally takes into itself the entire spermatozoon, the tail being wound in circular fashion within the cell body. The leucocytes, however, apparently accomplish most destruction by their dissolving or disintegrating action.

It seems that the migration of the leucocytes through the walls of the uterus and vagina, though not increased in extent, is accelerated by the act of copulation and the entire estrous process is shorter than in non-

copulated females. About six hours after copulation the third stage is in full development, while under virgin conditions a comparable stage is reached only after at least twelve hours from the time when copulation might have occurred. It may be said that copulation tends to hasten ovulation, or that the act itself may facilitate the bursting of the Graafian follicles, which is a very old conception.

The spermatic fluid of the guinea pig, especially that portion derived from the seminal vesicles, on entering the vagina of the female coagulates

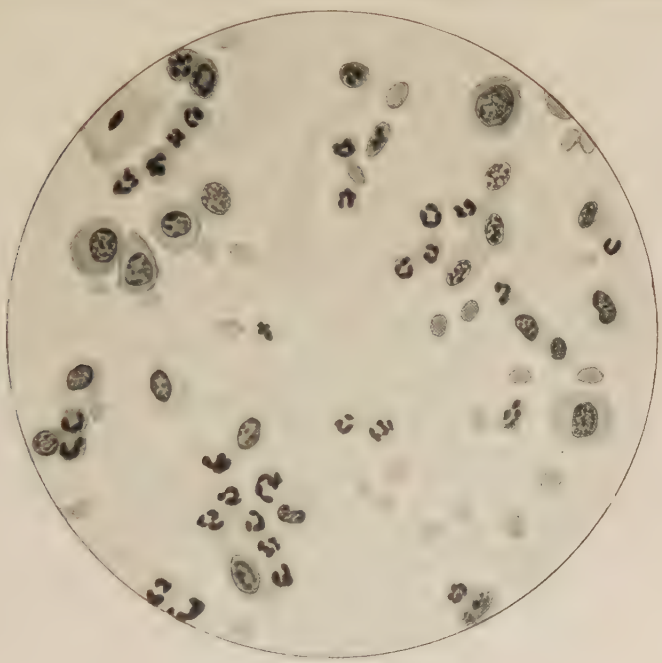


FIG. 530.—A smear showing the presence of red blood corpuscles in the vaginal fluid during the short "fourth stage." This slight hemorrhage does not always occur in the guinea pig.

to form the *bouchon vaginal*, a rigid plug, filling the lumen of the vagina. This plug prevents the outflow of the sperm after every copulation. Such a vaginal plug has been described in many species of rodents and seems in general to be characteristic of this class of mammals. It was first observed in the guinea pig by Leuckart in 1847. He correctly described it as a "Pfropf (plug)," formed by the coagulation of the secretion from the seminal vesicles and serving to fill the vagina and prevent the flowing out of the sperm after copulation.

Around this mass of coagulated seminal fluid the vaginal mucosa forms a close-fitting envelope, thus preventing its early dislocation. The envelope

serves to retain the plug in the vagina until the fourth stage of the estrous cycle, at which time the enveloping epithelium becomes completely separated from the vaginal wall by the dissolving effects of the leucocytes. The epithelium is now expelled as one continuous coat forming the cover around the vaginal plug instead of sloughing off in smaller pieces, as occurs during the fourth stage when a copulation has not occurred. However, the vaginal epithelium may occasionally be shed en masse without copulation.

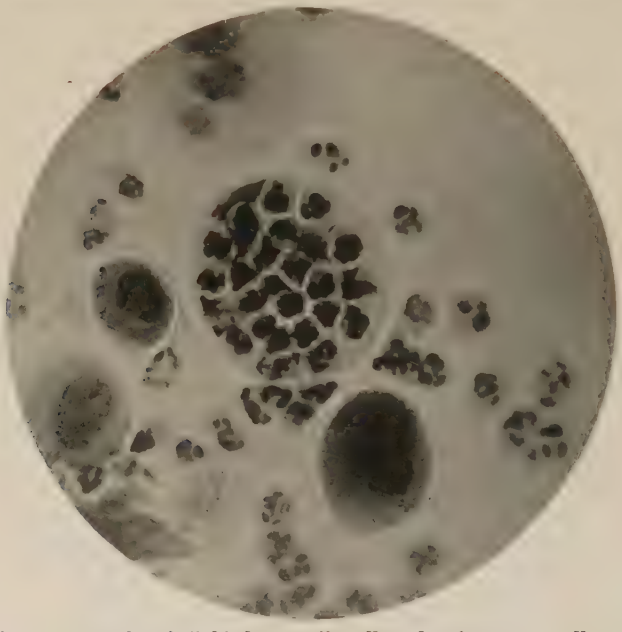


FIG. 531.—A photograph of "third stage" cells, showing one cell crowded with leucocytes.

III. CYCLICAL CHANGES IN THE UTERUS AND VAGINA GIVING RISE TO THE CELL CHANGES IN THE VAGINAL SMEAR

During the diestrum, or resting period, the uterus is lined by a layer of cuboidal ciliated epithelium. Figure 534 shows a section through the uterus at four and a half days after the last estrus. At this time the epithelial cells present a normal and vigorous aspect. No loss or breaking down is to be noticed. A few leucocytes are occasionally seen among the cells of the stroma, but never large numbers of them. Mitoses are not frequent at this time but they are to be noted occasionally.

When the heat period begins, the uterine epithelium loses its resting appearance (Figs. 535, 536 and 537). The cells become tall and columnar and are filled with mucus which is now secreted in abundant quantity.

The columnar cells appear closely pressed one against the other and the nuclei are forced into different levels in the various cells so as to give an appearance of several rows of nuclei. The epithelium thus takes on a pseudo-stratified arrangement. At the same time, a large number of leucocytes begin to migrate from the capillaries through the stroma and towards the lining epithelium (Figs. 535 and 536, leu.). The stroma itself is congested and possesses a more profuse circulation than usual.

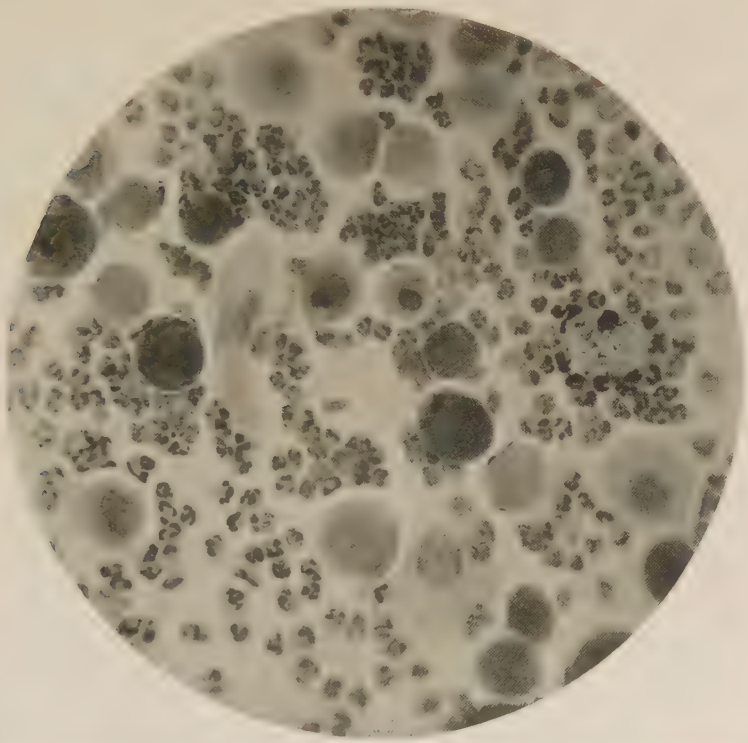


FIG. 532. A typical third stage smear showing the fading epithelial cells, probably being dissolved by the mass of leucocytes. A stage similar to that in Figure 529.

These conditions of the uterus are to be found in animals killed during the first phase of the estrous period, that is, when the vagina contains an abundant mucous fluid filled with desquamated epithelial cells. The smear of this fluid is illustrated by Figures 525 and 527.

When the second phase of the vaginal fluid appears (Figs. 526 and 528), the uterus presents another aspect. Leucocytes have accumulated in large numbers immediately beneath the epithelium, forming in this zone a perfect layer of leucocytes (Figs. 536 and 537). The stroma shows a more advanced degree of congestion.

During the third stage (smears, Figs. 529, 531 and 532) greater and greater numbers of leucocytes have penetrated into the epithelium, some of them making their way into the lumen of the uterus by passing between the epithelial cells. Other leucocytes actually enter the epithelial cells and lie in their interior (Figs. 538 and 539). Large vacuoles are to be seen between the epithelial cells, probably produced by the dissolving power of the leucocytes. Below the disintegrating epithelium hematomata are formed by the

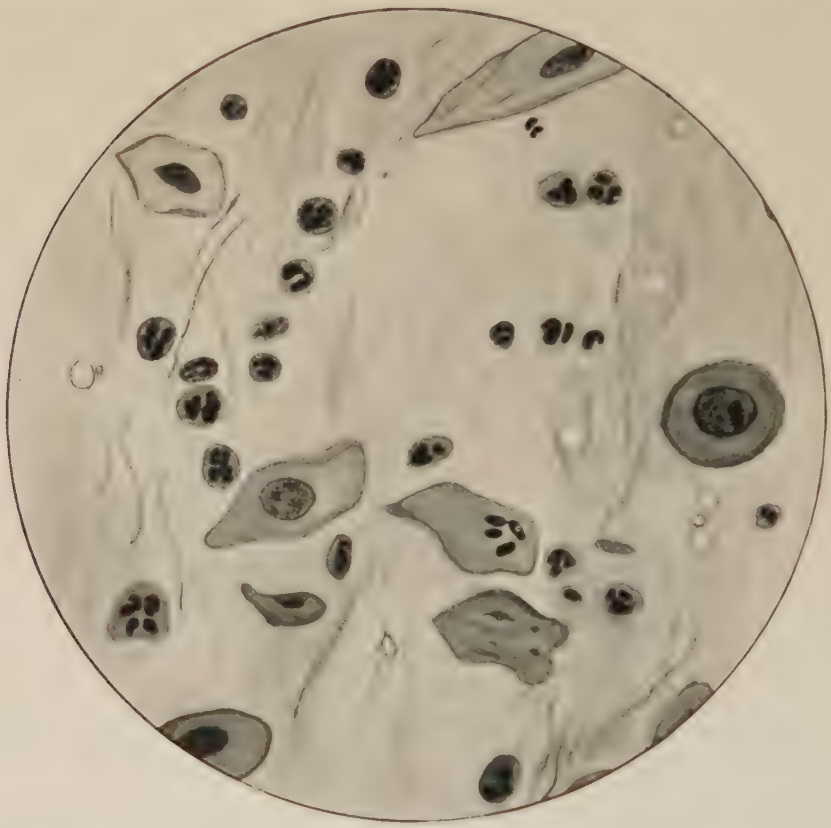
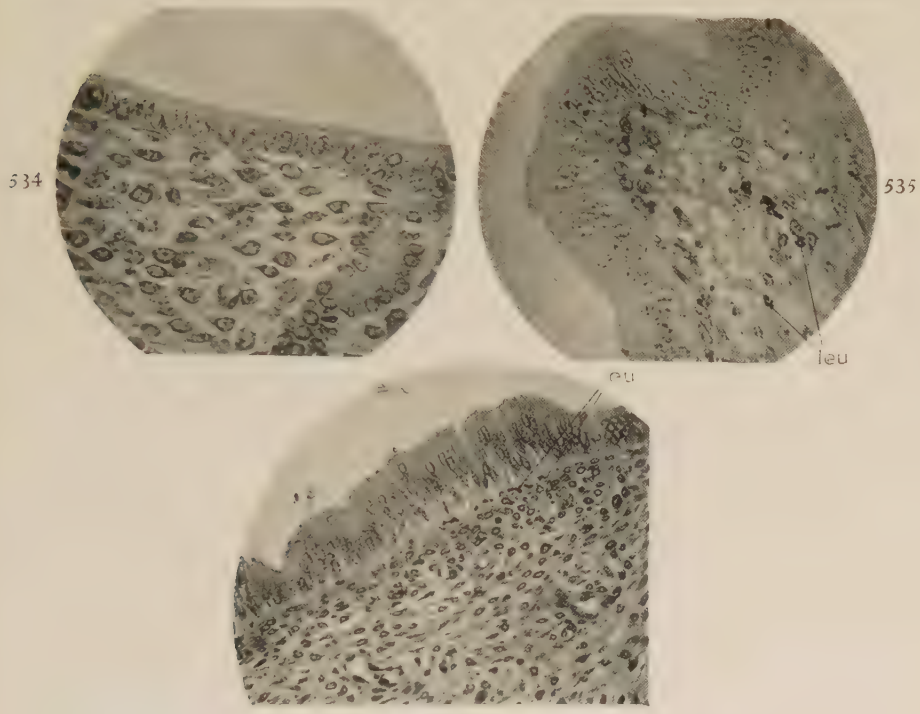


FIG. 533.—A drawing from a smear taken during the resting interval or metestrus. The vaginal fluid is very scant and the cells are thinly scattered through it. Leucocytes are still present; these are only absent from the first stage and early second stage smears.

congestion of the peripheral capillaries in the stroma. A leucocytosis somewhat similar to the above has been described by Heape, Königstein, Blair-Bell and others in the uteri of several mammals.

The broken-down epithelium remains until the regeneration begins during Stage 4. The reparation starts from the necks of the uterine glands

which have remained intact during the entire process of destruction. A few leucocytes are present between the epithelial cells of the uterine glands, but this small number apparently passes through the epithelium into the duct without injuring the epithelial cells. The stage of reparation corresponds to the fourth stage, that is, to the period when blood is sometimes seen in the vaginal fluid. This is not difficult to explain, since regeneration and the falling-off of the degenerated epithelium take place at the same time.



536

FIG. 534.—A photograph of a section of the resting uterus of a guinea pig during metestrum four and one-half days after estrus. The normal cuboidal ciliated epithelium is shown, and the smear in Figure 533 would be seen during this time.

FIG. 535.—Section showing the uterine epithelium during its excessive secretion of mucus and the beginning of leucocyte migration (leu, leucocytes).

FIG. 536.—Shows the same condition at a lower magnification and somewhat clearer. When the uterus is in this stage the vaginal fluid gives the typical first stage smear of Figures 525 and 526 (leu, leucotyes).

Regeneration of the uterine epithelium before the estrous flow had ceased is known for other mammals.

The wall of the vagina undergoes destructive changes somewhat similar to those in the uterus except that the desquamation of the vaginal epithelium does not occur in cell clumps or groups at the end of the third stage.

The epithelial cells of the vagina are shed singly but in increasing numbers from the beginning of the heat period up to the third stage. The cells which appear during the first stage come from the superficial layers of the stratified epithelium and near the outer part of the vagina, while during the second

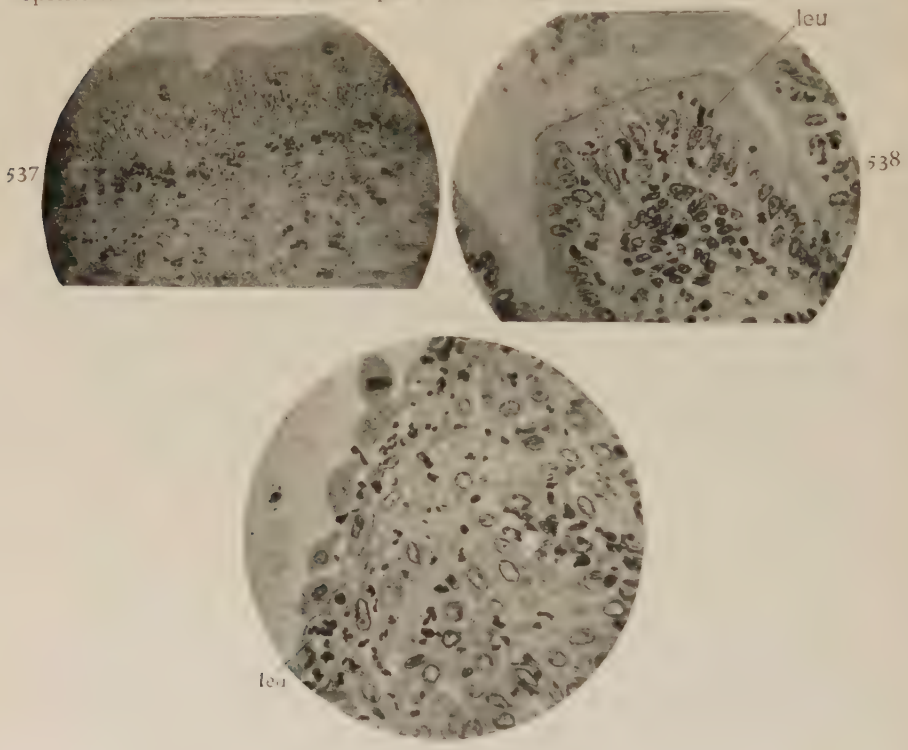


FIG. 537.—A section showing the condition of the uterine epithelium and the accumulation of numbers of leucocytes below the epithelium during the second stage of estrus. Corresponding smears of the vaginal fluid at this time are seen in Figure 526 and Figure 528 is actually from the same animal just before the uterus was removed.

FIG. 538.—A section through the uterus during the third stage of estrus showing the invasion of the epithelium by the migrating leucocytes. The epithelium is partially destroyed and vacuolized but still adheres to the underlying stroma which also contains leucocytes. A smear from this animal taken immediately before being killed is shown by Figures 531 and 532 (leu, leucocytes).

FIG. 539.—A section of the wall of the vagina from the same animal during, of course, the same third stage. The vaginal epithelium is also invaded by leucocytes and several cells are shown to contain leucocytes within their bodies. The epithelium here is being rapidly desquamated while the uterine epithelium in Figure 538 is disintegrating before being shed (leu, leucocytes).

stage the desquamated squamous cells are derived from the deeper layers of the vaginal epithelium. This statement does not include the cornified

cells from near the orifice, which are found between the first and second stages. The vaginal epithelium is also invaded by leucocytes. Leucocyte migration is decidedly vigorous during the third stage, about the same time that it occurs in the uterus. An innumerable army of polymorphonuclear leucocytes migrate into the vaginal epithelium and actually enter its more superficial cells by penetrating into their cell bodies (Fig. 539).

The beginning of desquamation before the massive arrival of the leucocytes would indicate that the primary cause of the desquamation is not the presence of the leucocytes. But, on the contrary, it is probably the presence of the altered and dying cells during the epithelial dehiscence which induces the extensive migration of leucocytes to this surface. The large epithelial cells of the vagina photographed in Figure 539 are the same cells which are to be observed in the vaginal fluid during the third stage (smears, Figs. 529, 531 and 532). A congestion of the capillaries of the mucosa also takes place in the vagina, and slight hemorrhages may occur as in the uterus, when the destruction of the stratified epithelium chances to extend into the tunica propria.

During the diestrum, or rest period, the desquamation of epithelium from the vagina does not stop completely and the scant vaginal fluid always contains some desquamated cells. At the same time, and probably connected with the shedding process, the exodus of leucocytes also continues, although to a very mild degree as compared with their migration during heat. The "intermenstrual fluid," therefore, always contains a few leucocytes and scattered epithelial cells (Fig. 533).

IV. THE OVARY AND THE CELLS OF THE VAGINAL SMEAR

Ovaries fixed during exact serial stages of the estrous cycle show that every change taking place in the uterus and the vagina corresponds with a change in the ovary. At the beginning of the first stage the ovaries possess large, ripe follicles. The nuclei of the eggs contained in the follicles are in a resting condition. The theca folliculi shows the beginning of a slight congestion. As this congestion becomes more and more pronounced, the first stage of estrus advances and by the beginning of the second stage the follicular congestion is highly developed (Fig. 540).

The ripe follicles break at about the end of the second or the beginning of the third stage. Figure 541 shows a follicle just broken at the commencement of the third stage. The examination of the vaginal smear gives exact information on the time when ovulation occurs. It may be recalled that at this time the active leucocytosis begins in the uterus and the vagina (compare Figs. 538 and 539). The ovaries are not omitted from this active migration of the leucocytes. A number of leucocytes are to be seen in the corpus luteum during its early development, but great numbers of leuco-

Fig. 540.—A photograph showing the vascular congestion of a ripe ovarian follicle from an animal killed during the second stage of estrus, by, blood vessel. Figure 538 shows a vaginal smear from this animal just before she was killed, and a section through the uterus is shown by Figure 537. All of the figures illustrate common second stage conditions immediately before the rupture of the follicle and ovulation take place.

Fig. 541.—A section from an ovary showing a Graffian follicle shortly after its rupture. The congestion in the theca folliculi is marked by, blood vessels. This animal was killed at the end of the second stage and just at the beginning of the appearance of numerous leucocytes in the smear.

Fig. 542.—An early corpus luteum from an ovary removed during the fourth stage. Vascularization of the corpus is seen to be proceeding from the periphery towards the center, by, blood vessels. This is taking place as the estrus period is just being finished.

cytes are to be found mainly in the atretic follicles, which are now becoming the seat of regressive and degenerative processes.

The ruptured follicles very quickly begin to undergo a reorganization and growth, resulting in the formation of the corpora lutea. Even during the third stage the corpus luteum already has a well-circumscribed body commencing its differentiation by the ingrowth of the vascular tissue of the theca folliculi into the hypertrophied follicular epithelium (Fig. 542). This condition is more advanced during the fourth stage, when reparation begins in the uterus. The well-formed mature corpus luteum exists on the day following estrus and the uterine epithelium soon returns to the typical resting condition of Figure 534.

V. THE NATURE OF THE ESTROUS PROCESS

After considering the above changes in the various parts of the female reproductive tract and the ovary, one is impressed by the striking similarity between these reactions and the ordinary inflammatory processes which follow acute irritation or infection. Few, if any, normal physiological changes in the mammalian body cause structural reactions so typically the same as those usually recognized under pathological conditions as does the process of ovulation. The estral reactions emphasize the fact that inflammatory processes are, after all, really physiological, that is, functional tissue responses for protective and regenerative purposes. Inflammation begins with an unusual dilatation of the small blood vessels in a limited region, a partial stagnation of blood results, and an active migration of leucocytes from the vessels into the surrounding tissues occurs. The effect of these conditions causes an accumulation of lymph and tissue juices in the region, and under the influence of the leucocytes pus is usually formed. The dilatation and congestion in the vessels around the enlarging Graafian follicle, and the same condition in the wall of the vagina and uterus during the first stage of estrus, is a physiological inflammation, and the vigorous migration of leucocytes into the tissues of these organs is a further step to be followed by a breaking-down and sloughing-off of the surface epithelia.

The cause of these changes in the present case is the periodic presence in the blood of an unusual chemical product or hormone produced by certain of the ovarian cells. This hormone seems to exert a selective effect upon the reproductive tract, yet other organs secondarily associated with reproduction, such as the mammary glands and even the sympathetic nervous system, are less acutely affected. The periodic arrival of this material in the ovary calls forth the acute inflammatory reaction rhythmically seen in the fertile female. The absence of the ovary or an interference with the occurrence of the hormone eliminates all of the estrous reactions. Modifications or slight interference with the production of the ovarian

material causes corresponding modifications in the uterine reactions and in the cellular composition of the vaginal smear. Thus the vaginal smear may serve as an indicator for any natural or experimental modification of the ovarian reaction; for example, during starvation and marked under-feeding the ovarian reaction is reduced and ovulation may be actually suppressed. The cellular composition of the vaginal smear then loses its periodic variations.

The influence of other glands of internal secretion upon the ovarian reaction as well as the effects of toxins and unusual chemical conditions within the body have been studied in recent years by the aid of the vaginal smear as an indicator of the ovarian state. This may be termed a cytological indicator and it is often as delicate and reliable in its response as one might expect a physical or chemical indicator to be.

VI. BIBLIOGRAPHY

Allen, E. 1922. The oestrous cycle in the mouse. *Am. J. Anat.*, **30**, 297.

Corner, G. W. 1921. *Cyclic changes in the ovaries and uterus of the sow, etc.* Carnegie Inst., Wash., Pub. 276.

——— 1923. *Ovulation and menstruation in Macacus rhesus.* Ibid., Pub. 332.

Hartman, Carl. 1923. The oestrous cycle in the opossum. *Am. J. Anat.*, **32**, 353.

Heape, W. 1894 and 1897. The menstruation of Semnopithecus, etc. *Phil. Trans.*, B., **185** (and **188**, 1897).

Loeb, L. 1911. The cyclical changes in the ovary of the guinea pig. *J. Morph.*, **22**.

——— 1914. The correlation between the cyclic changes in the uterus and ovaries in the guinea pig. *Biol. Bull.*, **27**, 1.

Long, J. A. 1919. The oestrous cycle in rats. *Anat. Record*, **15**, 352.

Long, J. A., and Evans, H. M. 1922. *The oestrous cycle in the rat and its associated phenomena.* Memoir, Univ. of Calif. Press.

Murphey, H. S. 1924. Studies of the oestrus or genital cycle of the ox. *Am. Vet. Med.*, **65**.

Papanicolaou, G. N. 1924. The production of certain distinct types of reactions by the use of ovarian extracts. *Proc. Soc. Exper. Biol. and Med.*, **22**, 106.

——— 1925. The diagnosis of early human pregnancy by the vaginal smear method. *Ibid.*, **22**, 436.

Papanicolaou, G. N., and Blau, N. F. 1923. The ovarian cystic fluid with special reference to its effect upon the reactions of the genital tract. *Proc. Soc. Exper. Biol. and Med.*, **21**, 164.

Papanicolaou, G. N., and Stockard, C. R. 1920. Effect of underfeeding on ovulation and the oestrous rhythm in guinea pigs. *Proc. Soc. Exper. Biol. and Med.*, **17**, 143.

——— 1922*a*. Morphology of cystic growths in the ovary and uterus of the guinea pig. *Ibid.*, **19**, 401.

——— 1922*b*. Experimental results bearing on the etiology of cystic growths in the ovary and uterus of the guinea pig. *Ibid.*, **19**, 402.

Stockard, C. R. 1925*a*. The general morphological and physiological importance of the oestrous problem. *Am. J. Anat.*, **32**, 277.

——— 1925*b*. The gonads and sex physiology. *Trans. Congress Am. Phys. & Surg.*, **13**.

Stockard, C. R., and Papanicolaou, G. N. 1917*a*. The existence of a typical oestrous cycle in the guinea pig: with a study of its histological and physiological changes. *Am. J. Anat.*, **22**, 225.

——— 1917*b*. A rhythmical "heat period" in the guinea pig. *Science*, N.S., **46**, 42.

——— 1919. The vaginal closure membrane, copulation, and the vaginal plug in the guinea-pig, with further considerations of the oestrous rhythm. *Biol. Bull.*, **37**, 222.

Tsu, Z-Y. 1924. Le rhythme vaginal chez la Lapine et ses relations avec le cycle oestrien de l'ovaire. *Ed. Medical Fac.*, Strasbourg.

Van Herwerden, M. 1906. Beitrag zur Kenntnis der menstruellen Cyclus. *Monatschr. f. Geburts. u. Gynak.*, **24**.

Section XXXIV

THE CYTOLOGY OF THE MAMMARY GLAND

CONTENTS

Section XXXIV

	PAGE
I. Structure of Mammary Gland	1178
II. Life Cycle of Mammary Gland	1180
III. Mammary Gland during Sexual Cycle	1185
IV. Growth of Mammary Gland during Pregnancy	1187
V. Secretion of Mammary Gland	1188
1. Mechanism of secretion in mammary gland	1192
2. Retrogression of mammary gland	1195
VI. Changes in Stroma of Mammary Gland	1198
VII. Mammary Gland under Experimental and Pathological Conditions	1201
VIII. Bibliography	1204

Section XXXIV

THE CYTOLOGY OF THE MAMMARY GLAND

LEO LOEB

IN common with other tissues of mammalian organisms, the mammary gland develops, reaches an adult stage and then undergoes certain degenerative changes characteristic of old age. There is, however, superimposed upon this principal cycle a series of shorter cyclic changes which in their particular sequence are found only in the mammary gland, although in certain other respects it shares these cycles with the other individual organs which compose the female generative organ system, of which the mammary gland forms a part. The mammary gland belongs to the group of organs which undergo cyclic changes under the influence of stimuli originating in the ovary.

In common with a wider group of tissues or organs, the mammary gland has a proliferative activity, thus differing from certain stationary non-proliferating tissues, such as the system of ganglia cells. However, it is unlike some of the other proliferating tissues as, for instance, the epidermis, in that in the latter the conditions leading to proliferation seem to be mainly of an endogenous nature, while in the mammary gland they are largely caused by exogenous hormone action, a characteristic shared also by the uterus and vagina.

However, joined to the cyclic periods of rest and proliferation, are periods of secretion and retrogression, followed anew by phases of proliferative and secretory activity, and all these phases are component parts of the cycle of the mammary gland.

Participating in the activity of the mammary gland cells and correlated with it are changes in the surrounding stroma. We shall have to analyze the character of these correlative changes and in particular we shall have to inquire whether the stroma of the mammary gland is individualized in the same sense as, for instance, the stroma of the uterine mucosa, or whether the properties of this stroma are the same as the ordinary connective tissue elements, and what some of these properties are. In addition to these typical changes we must include in our analysis certain other transformations which occur in the mammary gland more rarely, under special experimental or spontaneous pathological conditions; they reveal potentialities of the mammary gland cells which, while normally dormant, are inherent in the constitution of these cells.

As in the case of all other tissues, so also in the mammary gland the life phenomena depend upon the cooperation of inner constitutional factors inherent in the cells and of factors external to these cells and originating

in the environment of the tissue. These specific and non-specific conditions in the inner environment acting on the cells and producing the typical structural, metabolic and functional changes in the tissues are largely known. We also understand fairly well the result of this interaction, namely, the cycle to which we referred above. However, we are entirely ignorant of the mode of interaction between these internal and external sets of factors within the cell upon which these specific results depend. In so far, then, a cytology of the mammary gland must necessarily be deficient in some very essential respects.

Before we enter into a consideration of these various problems, it will be necessary to give a brief account of the mechanism of the female sexual cycle which so largely determines the changes in cell activity in the mammary gland.

In accordance with conclusions at which we arrived in former years, we can divide the sexual cycle into various phases, the first of which is the follicular phase caused by the maturing follicle. In this phase we find typical changes in the uterus, growth processes in the mammary gland and, as Stockard and Papanicolaou (1917) have observed, also in the vagina. This follicular phase associated with heat and spontaneous ovulation in certain animals, in contrast to others, as, for example, the rabbit, in which ovulation occurs only in case copulation has taken place previously) is followed by a brief period of rest. But this inactivity is soon succeeded with or without pregnancy by the lutein phase in animals which ovulate spontaneously. In the rabbit it occurs only if copulation has previously taken place. In the latter animal the lutein phase can also be made to occur independently of pregnancy if subsequent to copulation the Fallopian tubes have been ligated, a method first used by us, or by the previous ligation of the spermatic cord of the copulating male, a modification of our method, subsequently used by Ancel and Bouin. The lutein phase is characterized by the action of hormones given off by the corpus luteum. Our experiments showed that such a hormone causes slight growth processes in the uterine mucosa; that it furthermore sensitizes the latter in such a way that it is now able to produce the maternal placenta under the stimulus of the fertilized ovum, or multiple placentomata under the influence of mechanical stimuli. In addition, it causes growth processes in the mammary gland which may become effective either at once, as in the rabbit (Ancel and Bouin, 1909, 1911, 1912; Hammond, 1917), or after a prolonged period of latency, as in the guinea pig (Loeb and Hesselberg, 1917). The character of the cycle, which Long and Evans (1922), as well as Ishii, observed in the rat, and Allen (1923) in the mouse, is not contradictory to this interpretation, but can be readily harmonized with it if we consider that in these latter species the lutein phase is, under ordinary conditions, rudimentary because of the short duration of the cycle; but the typical effects of the corpus luteum are obtainable here, also, under certain conditions.

In addition, the corpus luteum has a further effect in preventing ovulation and thus indirectly of determining the periodicity of the sexual cycle, which latter is directly determined by the time required for the maturation of the ovarian follicle (Loeb, 1911). Cessation of function of the corpus luteum is a necessary condition for the rupture, but not for the maturation, of the ovarian follicle.

If pregnancy follows copulation, the life of the corpus luteum is prolonged and its growth intensified through the presence of the embryo, and in part at least through the development of the maternal placenta. In consequence of this increased and prolonged

activity of the corpus luteum the mammary gland shows an increased and prolonged growth which, after cessation of the activity of the corpus luteum, is followed by secretion and subsequently by retrogressive changes. In the sensitized uterus, the fertilized egg causes the formation of the maternal placenta and indirectly through its action on the corpus luteum prevents ovulation, but not necessarily the growth and beginning maturation of the ovarian follicles. Accordingly we found that, if during pregnancy the corpora lutea are extirpated, a new ovulation can be effected in the guinea pig, notwithstanding the presence in the uterus of living embryos. However, we further observed that this new ovulation is not followed during pregnancy by typical cyclic changes in the uterus, the uterus having become refractory to these stimuli under these conditions. It is, therefore, the uterus which is inactive during pregnancy once the placenta has been produced, but not, as is usually assumed, necessarily the ovary. These facts apply in particular to the guinea pig.

There is, as we found in the guinea pig, another method besides pregnancy which allows us to extend very considerably the duration of life and growth of the corpus luteum; namely, the extirpation of the uterus a few days after ovulation. It is a peculiar phenomenon that pregnancy as well as lack of uterus, two conditions so different from each other, should both have a similar effect on the corpus luteum. After hysterectomy, heat and ovulation are therefore suspended in the guinea pig, at least for a long period of time, and it seems, furthermore, that in hysterectomized guinea pigs, because of the long-continued preservation of the corpus luteum, the growth of the mammary gland may be prolonged. But this latter point needs further investigation.

A more extended analysis of the cycle has been made possible through the discovery by Frank (1911, 1912), and especially by Allen, Pratt and Doisy (1923), that by the injection of follicular fluid certain changes in uterus and vagina can be produced experimentally which, according to our interpretation, are characteristic of the follicular phase. However, the further conclusion of these authors, that it is this hormone alone which causes all activities of the sexual organ system during the cycle, does not seem to us in agreement with the facts. On the contrary, the evidence seems to us definitely to point to the conclusion that in addition to the follicular hormone there is at least one other hormone given off by the corpus luteum, but there are probably several others and all quite distinct from the follicular hormone.

A complication of the typical character of the sexual cycle is observed in apes and in women. Here a periodic menstruation is added to the other rhythmic changes which we mentioned. As to the position of menstruation in the cycle and as to the analogue of it in lower animals, there has been much dispute. As late as in 1909 Ancel and Bouin, and in 1911 R. T. Frank, held that it corresponds to the proestral period of the cycle. However, there can be little doubt that it occurs at the time of cessation of growth and beginning degeneration of the periodic corpus luteum in cases in which fertilization of the ovum does not occur; in such cases the egg does not develop into an embryo and does not cause the production of a placenta and thus the prolongation of life of the corpus luteum which normally occurs in pregnancy cannot take place.

This discussion will, we hope, explain how the mammary gland placed in the generative organ system is in its activities dependent upon and connected with the activities of the ovary and uterus; the ovary being a composite organ, which includes follicles as well as corpus luteum, primarily determines the sexual cycle, of which the mammary gland in its functioning is a part.

But before discussing the changes of the mammary gland in the sexual cycle, we shall first study its ordinary life cycle after having briefly described its elementary structure. This will be followed by an analysis

first of growth and of secretion of this organ, without and with accompanying pregnancy, and subsequently of the retrogressive changes following growth and secretion. We shall then study the relationship between parenchyma and stroma in this gland and the general characteristics of both kinds of tissue as revealed by experimental methods, and in the end shall consider some properties of these tissues which are ordinarily hidden, but are revealed through certain pathological changes as being always potentially present. These latter changes lead to increased growth processes and are the expression of a stimulated state of the gland, either of a temporary or of a permanent character.

I. STRUCTURE OF THE MAMMARY GLAND

The mammary gland consists of a system of epithelial ducts which show a degree of ramification varying according to the character of the inner environment, and in particular of the growth substances which act on them (Fig. 543). The vesicular enlargements of the smaller ducts, or terminal tubules, constitute the alveoli. A basement membrane demarcates both ducts and alveoli from the surrounding stroma. As far as their morphology is concerned, the ducts and alveoli differ in the main in the number of cell rows which line both of these structures. Those parts of the ducts which are nearest the epidermis, from which they originated, are still lined with squamous epithelium, but the remaining parts consist of two rows of cells of epithelial origin, the outer row lower than the inner one. The former is made up of flat or spindle cells, the long axes of which are directed perpendicularly to the direction of the inner layer, which consists of cuboidal or cylindrical cells with well-developed oval nuclei. In the alveoli, on the other hand, there is essentially only one epithelial cell layer, the structure of which differs under different conditions; but around the alveoli there may be found also a small number of elongated stellate cells, sending out long fine processes, the so-called "knob" or basket cells.

The transition between the ducts and the alveoli, however, is not an abrupt one; on the contrary, there is a close relationship between them, the differentiation which takes place in the ducts and which leads eventually to the development of the alveoli being very gradual. In women it has been observed that alveoli develop, as a rule, only during pregnancy when the proliferative activity of the gland reaches its greatest intensity, but in certain of the lower animals alveoli may form during active growth processes occurring under various conditions other than pregnancy. Apparently this relationship between terminal tubules and alveoli is not only structural in nature, but it is physiological also. The terminal tubules may at certain periods secrete a fluid resembling colostrum. This secretory activity, as always, is associated with preceding growth processes and takes place

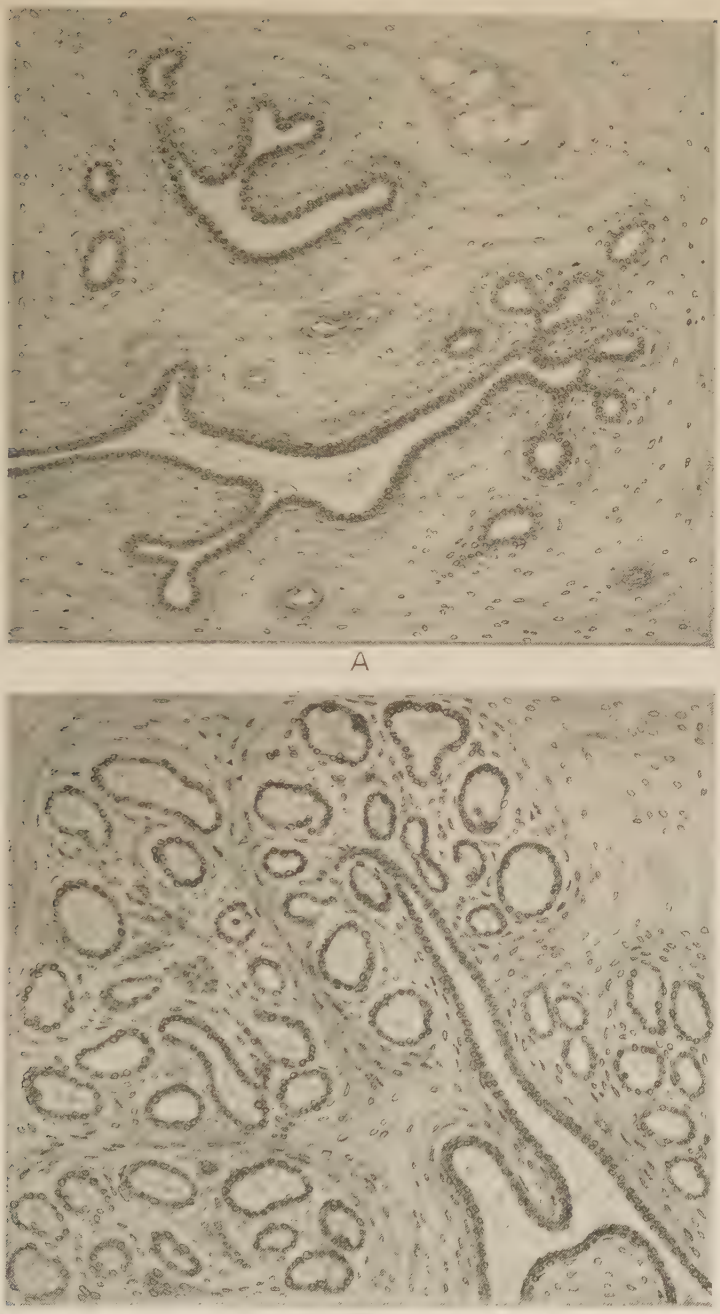


FIG. 543.—Resting (A) and proliferating (B) mammary glands of the rabbit (during lutein phase). × 150. (After Ancel and Bouin, 1911.)

at a time when alveoli are not yet formed, as in the stimulated gland of newborn children. Furthermore, under certain other conditions fat droplets have been found in the epithelium of the ducts. We must, however, in this instance consider the possibility that not all the fat noted in these ducts originated in them, being synthesized in these cells from the constituent substances carried to them through the circulation; but that fat secreted by the alveoli and stagnating in the lumen of smaller ducts may enter the epithelium of the latter from the side of the lumen: an absorptive in addition to a secretory activity must here be taken into account.

An additional indication of the close relationship between the terminal ducts and the alveoli is found in the relatively cellular, non-fibrous character of the stroma surrounding these ducts, in contrast with the fibrous stroma around the larger ducts. This difference again gives evidence of a more active metabolism in the terminal duct epithelium which makes it akin to the epithelium of the alveoli. There is, then, a gradation of structure, metabolism and function from the most peripheral excretory ducts to the central terminal ducts and alveoli.

A peculiar problem is presented by the outer layer of cells lining the ducts and by the corresponding but more isolated cells found around the alveoli. At least the continuous outer layer of cells forming parts of the ducts is of epithelial origin. According to Benda (1894) and other authors they are held to be smooth muscle cells in which a definite fibrillation can be made out similar to that seen in other smooth muscle cells and which give also staining reactions considered characteristic of the latter. Their function is believed to be that of pressing out through their contraction the secreted material from the ducts. In accordance with this view of their character and origin, they are designated as myoepithelium. Other observers, however, doubt that the smooth muscle cells which are found in or around these ducts are the outer epithelial layer of the ducts; they assume instead that they are cells surrounding the capillaries of non-epithelial origin, corresponding to ordinary mesenchyme which somehow have become closely approximated to the ducts and the alveoli. Such a conception has been expressed more recently by Dieckmann (1925) and Krompecher.

II. THE LIFE CYCLE OF THE MAMMARY GLAND

The mammary gland was formerly thought to be the homologue of sebaceous glands, and the milk production a process analogous to the activity of the latter. However, it is now generally believed that the mammary gland corresponds to the sweat glands (Benda, 1894; Eggeling, 1904; E. Bresslau, 1902). It takes its origin in invaginations from the surface epithelium, dipping down into the underlying connective tissue, first as solid ducts which subsequently form a lumen; some branches are developed in the

underlying fatty tissue. During the prepubertal period a very slow growth takes place in this duct system through mitotic division; but mitoses are very rare at this time. The growth consists largely in an elongation of the ducts as new lateral branches are formed only to a limited extent. The duration of the prepubertal period varies greatly in different species: in the rabbit, it may last from six to nine months; in the rat, about eight to nine weeks; and in woman, it usually ends in the second decade of life, though it may be found as late as nineteen years after birth.

If we compare the development of the mammary gland in the male and female rat, no difference is found between the two sexes up to the age of five weeks. At that time a more marked growth and ramification of the milk ducts takes place in the female (Myers, 1916, 1917, 1919) and from now on the gland remains better developed in the latter than in the male, until the changes taking place at puberty increase still more this difference between the two sexes.

If we inquire into the cause of this prepubertal growth, it may be assumed to be due in part to general growth factors, presumably growth substances acting equally in both sexes. Superimposed on this general effect is possibly a specific growth promoting effect of the ovary on the female gland. If the ovary is extirpated early in life, the puberty growth of the mamma is prevented, and, in addition, it seems that even a partial absorption of the terminal portions of ducts already formed may take place. Autotransplantation of the gonad may restore the normal growth (Knauer, 1900; Kehrer, 1901 and Halban, 1905). Which constituent of the ovary is responsible for this additional growth in the female before puberty is uncertain; it appears most likely that the small and medium-sized follicles exert a certain effect. In particular, it is not clear at present why such a discontinuous growth should occur as that which Myers (1919) described as occurring in the rat. In order to obtain further insight into these processes, it will be necessary to compare the changes in ovary and mamma during this period. Whether the small and medium follicles of the ovary really can influence the mammary gland must still be considered uncertain at present and further investigations in this respect are indicated. With regard to the arrested growth of the male gland it is possible that the developing testicle may exert a certain inhibiting effect.

Within the first week after birth, some secretion takes place in the mammary gland of the newly born animal or child. It is not true milk which is thus produced, but in the child the secretion contains fat and resembles colostrum; in the rat, fat droplets do not seem to occur at this time. As to the cause of this secretion we must assume that it is due to the same condition which causes secretion also in the mother. Either corpus luteum or the maturing follicle in the ovary of the mother stimulates probably a slight proliferation in the gland of the fetus directly preceding birth and,

as the result of the sudden cessation of this hormone action, changes, which lead to secretion, take place in the gland of the newborn. This secretion is found in the terminal milk ducts rather than in typical alveoli. The secreted material may be retained for a long time in the ducts and distend some of them.

In this connection it is of interest that, according to Loewe, the follicular hormone can be demonstrated in the venous blood of the umbilical vein, indicating that this substance passes from the maternal blood through the

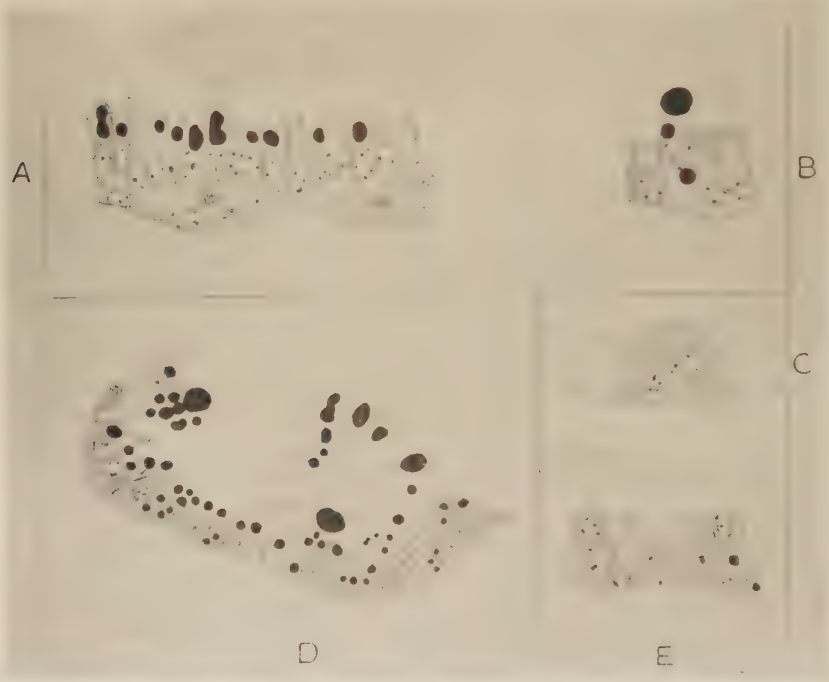


FIG. 544.—A and B, mammary gland secretion in a newborn child. C, secreting cell with three nuclei. D, decapitation of secreting cells. (After Brouha, 1905.)

placenta to the fetus. During the prepubertal period a development of the ducts takes place, but no real alveoli are formed; but with the approaching puberty in the rat, an enlargement of the terminal ducts may occur, a condition approaching the formation of alveoli.

With the onset of puberty a marked growth of the mammary gland generally occurs. The ducts branch dichotomously and mitotic division is more active. As to the formation of true alveoli lined with one layer of epithelium, the statements of various authors differ. In women this seems to be lacking at this period, while in the rabbit, according to Ancel and

Bouin, (1911) it occurs to a moderate extent. With the completion of growth, some fat droplets may appear in the apical parts of the cells of the terminal structures, a process corresponding to a rudimentary milk secretion. Accompanying this development of the gland and representing an integral factor in it, a marked formation of capillaries takes place around these terminal ducts and alveoli.

The entire growth process during the period of puberty depends upon the action of the follicular hormone; it may be said to correspond to the first —the follicular—phase in the sexual cycle. But in certain animals this phase may be closely followed by the second, or lutein phase. During the latter, a very marked additional growth takes place as is, for instance, the case in marsupials (O'Donoghue, 1912; Hartmann, 1923), in which a spontaneous ovulation occurs leading to the formation of a corpus luteum. In other animals with a spontaneous ovulation, the lutein phase in the growth of the mammary gland is lacking, either because the effect of the lutein hormone on the mammary gland is retarded, as in the guinea pig (Loeb and Hesselberg, 1917), or because the duration of the lutein phase in the cycle is very short, as in the rat and the mouse. In woman the condition seems to approach that found in the guinea pig; a definite lutein action on the mammary gland is apparently lacking during the ordinary cycle, or at least it does not occur regularly. In animals which do not ovulate spontaneously, as in the rabbit, no corpus luteum is usually formed after heat under natural conditions, and it is only the follicular phase in the cycle which is represented.

These statements also hold good in regard to the changes which take place between the periods of puberty and of the first pregnancy. If the latter does not follow the onset of puberty at once, there is inserted in the life of the animal a series of sexual cycles in which a limited development of the mammary gland occurs owing to the action of the follicular hormone alone, or to the successive action of the latter and of the lutein hormone, according to the kind of animals considered. Some retrogressive changes seem to follow each period of growth, but they are apparently not sufficiently pronounced to undo entirely the work accomplished during the growth rhythms. Thus as a whole the mammary gland enlarges during this period also, apparently to a different extent in different individuals. But a careful study of this phase of the mammary cycle has apparently not yet been made and some of our conclusions are therefore inferences rather than definitely observed facts. However, we can be certain that castration prevents the occurrence of these pubertal as well as postpubertal changes, because it precludes the development of maturing follicles, in particular of their granulosa, and of the corpora lutea.

If for the present we pass over the periods of pregnancy and the associated states of lactation and retrogression and consider the end stage of

the life cycle, the senile period of the mammary gland, we must rely mainly on studies of the human organ; but as far as we know conditions are similar, at least in certain animals, as, for example, the dog. This stage is characterized by a marked retrogression of the parenchyma. The alveoli have disappeared. The ducts also diminish in number and fat may appear in their epithelial cells. The epithelium may become lost, and the surrounding fibrous tissue may obliterate the lumina of the ducts, their previous situation being indicated by strands of elastic tissue. But in a number of cases there are found associated with these retrogressions, proliferative changes consisting in cyst formation and increase in the rows of epithelium. Some of the cysts are characterized by a pale cytoplasm. The stroma of the gland is dense, especially around the ducts, but in time it seems to be partly absorbed between the ducts and to become replaced by fat tissue. Vascularization diminishes; the elastic tissue, especially around milk ducts, but also around vessels and elsewhere, is usually markedly developed. Thus in old age the gland not only returns to a resting condition, but in addition shows peculiarities of a degenerative, and sometimes of a proliferative character. These alterations are due to a combination of lack of ovarian function and of general metabolic changes characteristic of old age.

Before entering into a discussion of the sexual cycle which is superimposed upon the life cycle of the female mammary gland, we shall refer briefly to the factors determining the behavior of the breast in the male animal. As we have stated, here the gland remains normally quite rudimentary; it consists of a limited number of milk ducts which show only a very restricted growth and branching, as compared with the female gland. This difference sets in even before the period of puberty; it does not, therefore, depend entirely on the action of mature ovarian follicles, but either on the lack of a limited hormone action originating in the smaller follicles, or on an inhibiting action of the testicle. That the testicle may exert an inhibiting effect not only on the development of the follicles of the transplanted ovary, but also on the action of the follicular hormone on the mammary gland has been made probable, especially through the experiments of Lipschütz and Voss (1924). On the other hand, the male breast has the potentiality of the same pronounced development as the female gland, provided it is furnished with the necessary hormones. An ovary transplanted into a castrated male may supply this substance even more effectively than in a castrated female (Lipschütz); on the other hand, injection of the follicular hormone seems to be much less potent in the male as the experiments of Vintemberger (1925) would indicate. A similar hormonal action can apparently be exerted also by non-descended testicles, consisting merely of interstitial tissue and Sertoli cells (and perhaps not well-differentiated spermatogonia, according to Oslund), at least in the guinea pig, as our observations seem to indicate. It may very well be that the further development and activity of the spermatogonia inhibit the factors promoting growth of the mammary gland, while Sertoli cells or interstitial testicular tissue may favor it.

An interesting condition has been observed in male pseudo-hermaphrodites where a development of the mammary gland may occur in certain cases. Here the interpretation is difficult because, in addition to the state of the sex glands, we must consider a possible tendency to maleness or femaleness inherent in the soma as a possible complicating factor.

III. THE MAMMARY GLAND DURING THE SEXUAL (ESTROUS) CYCLE

We have already stated that two growth phases may be recognized in the mammary gland during the ordinary sexual cycle which is not complicated by the occurrence of pregnancy. Preceding and directly following ovulation a growth takes place which depends upon a secretion given off by the maturing and mature follicles; this has been demonstrated by us in the guinea pig and it is apparently found also in rabbit, rat (Sutter 1919, 1921) and mouse. The growth of the mammary gland after transplantation of ovarian tissue into castrated male or female animals depends also on the development of these follicles (Athias, Lipschütz). As we stated above, injection of follicular fluid in castrated rabbits leads, according to Vintemberger (1925), only to a limited growth of the mamma; less apparently than the proliferation which follows transplantation of the ovary. This may be another instance of a condition which we noted in the case of the corpus luteum; namely, that repeated injections of a hormone can, in certain cases, only to a limited extent, or not at all, imitate the effect of the constant secretory activity of an organ. This transitory growth of the mammary gland in the follicular phase seems to be followed by slight secretory and retrogressive changes.

With the onset of the lutein phase a marked proliferation of the gland sets in, in the rabbit (Ancel and Bouin, 1909, 1911) and also in marsupials (O'Donoghue, 1912; Hartmann, 1923). There can be no doubt that this growth is due to a hormone given off by the corpus luteum, a conclusion first arrived at by Ancel and Bouin (1909) in the case of the mammary gland of the rabbit and confirmed and further analyzed by Hammond (1917, 1925). In the rat and mouse this growth during the lutein phase apparently does not occur; this is probably due to the rudimentary development of this stage of the cycle in these animals. From the brief statements available in the literature we must, on the contrary, assume that retrogressive changes take place in the breast during this period. In the case of the guinea pig, we found the lutein phase in the mammary gland to be lacking or almost lacking. Only if we prolong experimentally the duration of the sexual cycle, through production of placentomata with which is associated a prolonged life of the corpus luteum, may we observe growth processes in the mammary gland. Extirpation of the corpora lutea seems to prevent this phase of proliferative activity but it accelerates the onset of the follicular growth phase through the removal of the inhibiting action of the corpus luteum on ovulation. If we check the full development of follicles through underfeeding and subsequently hypotypical ovaries are produced, both growth phases are lacking, just as after castration.

These differences between the response of the mammary gland to growth stimuli in different species may perhaps be due to differences in the character of the mammary gland in these species. There can be no doubt that otherwise the corpus luteum, as far as the effects on formation of placenta and placentomata and on cyclic growth of the uterus and the regulation of periodicity of the cycle are concerned, is as active in the guinea pig as in the rabbit.

As we have stated, the lutein phase of growth seems to be present, also, in the guinea pig but it is delayed; a cumulative effect of the corpus luteum hormone is apparently necessary to produce changes which in the rabbit take place more readily. We found that it is possible to prolong the life of the corpus luteum in the guinea pig to a remarkable extent, and correspondingly to prolong the sexual cycle, by extirpation of the uterus a few days after ovulation. In this case we observed continued growth processes in the mammary gland during the latter part of the cycle which was thus very much prolonged, and we attributed this condition to the cumulative action of the corpus luteum. However, our observations on the mammary gland in these cases were made in animals of various ages and it will be necessary to repeat them in a larger number of young virgin guinea pigs before we can draw the conclusion definitely that in such cases the prolonged life of the corpus luteum increases the growth of the mammary gland.

In rabbits, Ancel and Bouin (1909, 1911) found the proliferation of the mammary gland to be most active towards the end of the first week following ovulation. Towards the end of the second week, a gradual cessation of growth takes place; then some secretion processes occur and very soon retrogressive changes take place.

As to the cyclic changes in the mammary gland of woman during the estrous cycle, the conclusions at which different investigators have arrived are contradictory. We can be certain that some cyclic changes are present. Thus during the latter part of the interval and during the premenstrual period, hyperemia and a succulent condition of the intraalveolar stroma occur (Dieckmann, 1925). There may also perhaps be some growth processes in the milk ducts. During the postmenstrual period and in the first part of the interval the stroma and the basement membrane become dense. Some authors believe that especially in the praemenstruum a new formation of alveoli and lobules may take place, which later disappear; others deny these changes in the parenchyma. It is conceivable that stroma and glandular alterations in the premenstrual period are due to the corpus luteum action. With the beginning degeneration of the latter, menstruation sets in. As to the follicular growth phase of the mammary gland, this has not yet been established in this cycle, owing perhaps to the difficulty of determining the occurrence of the follicular phase satisfactorily in the case of woman.

IV. GROWTH OF THE MAMMARY GLAND DURING PREGNANCY

During pregnancy the growth of the mammary gland depends again upon the stimulating substance given off by the corpus luteum. Inasmuch as the latter organ usually has during this period a longer duration of life and growth than in the ordinary estrous cycle, the mammary gland develops more markedly during pregnancy. This is very noticeable in the guinea pig where we found no pronounced growth during the estrous cycle, while during pregnancy the mammary gland produces typical alveoli, and mitotic division is observed in both ducts and alveoli. Also in rabbits the growth extends over a longer period in the pregnant animal. On the other hand, in marsupials, where corpora lutea behave in a similar manner during the ordinary sexual cycle and during pregnancy, mammary glands likewise behave in the same manner in both conditions. Again, we find species differences in regard to the responsiveness of the gland tissue; thus in the rabbit the growth begins very soon after fertilization, while in the guinea pig a relatively long latent period precedes the growth, as is the case also during the sexual cycle. There seems to be some relation between the duration of pregnancy and the reactivity of the mammary gland and likewise between duration of pregnancy and length of the growth period of the mammary gland.

However, there are indications that the same characteristic growth curve which we find in other conditions (Robertson, 1922), showing a maximum of growth in the center of the curve is also observed in the breast during pregnancy. During the growth itself substances or mechanisms develop which tend to inhibit growth. Thus we find that although the buds of the terminal ducts, which develop into alveoli, have still the power to divide mitotically, they do not produce a second set of alveoli. On the contrary toward the latter part of pregnancy, the mitotic proliferation seems to diminish and some processes of secretion set in, although the latter are apparently limited in intensity as long as the corpus luteum is functioning. In cases in which an active milk-like secretion is seen before labor, there are presumably already at this time degenerative changes in the corpus luteum. It is very likely that the growth stimulus given off by the corpus luteum inhibits also to a certain extent secretory processes. The cause of this gradually decreasing growth energy in the gland tissue may possibly be found in the changes taking place in the gland cells in the course of development which somehow make these cells less able to divide and more prone to secrete, a process probably analogous to differentiation which so often accompanies growth processes. How far a decrease in energy of blood circulation within the gland plays a part in this differentiating process is uncertain. Ancel and Bouin (1911, 1912) attributed these secretory processes in the latter part of pregnancy to the action of a special organ,

the myometrial gland which, they believed, developed in the uterine muscle tissue at that period. They distinguished, therefore, a proliferative and a glandular phase in the breast during pregnancy. However, these two periods do not seem to be very sharply delineated and, furthermore, it has been shown that those structures which have been designated by Ancel and Bouin (1911, 1912) as myometrial glands are not found regularly and that they do not have the significance attributed to them.

That it is the corpus luteum which provides the growth stimulus for the mammary gland during pregnancy is further shown by the extirpation of the ovaries in the guinea pig during the earlier period of pregnancy. This causes a cessation of growth of the gland even at a time when pregnancy still continues. Extirpation of the corpora lutea in the pregnant guinea pig, on the other hand, induces a new ovulation notwithstanding the presence of pregnancy and with it the follicular phase of proliferation in the breast, although the lutein phase would thus be eliminated temporarily. As we have stated repeatedly, the corpus luteum does not prevent the maturation of the follicles, but it prevents only ovulation and the changes directly preceding the latter process. During pregnancy in the guinea pig there are no signs of a hypotypical state; there is, on the contrary, the usual far-going development of ovarian follicles. A hypotypical condition of the ovaries is, however, found in some other species associated with a marked development of the corpus luteum. Again it can only be a hormone of the latter organ which in such cases stimulates the growth of the mammary gland. Removal of the fetuses from the uterus of the pregnant rabbit causes cessation of the growth of the mammary gland through the effect of this process on the corpus luteum, although the placenta may still be retained for some time (Hammond, 1925).

As to the relation of growth to circulation, it is the same as in other instances in which a hormone is responsible for proliferative phenomena, as, for instance, in the case of placentomata. Growth is associated with an intensified circulation and an increased blood supply. A good supply, in particular of oxygen and certain foodstuffs, is necessary for a very active cell proliferation, but it is not the primary and specific factor setting up growth. The latter is due to the specific growth substance which initiates growth processes. These initial growth phenomena, then, secondarily affect the capillaries which become dilated and send out new branches around newly forming tissue.

V. SECRETION OF THE MAMMARY GLAND

In general, the mammary gland begins to produce a characteristic secretion, milk or a material more or less closely resembling milk, as soon as the growth substances have produced a certain amount of tissue and a sudden cessation of the proliferative activity has taken place. This cessation of growth processes is again conditioned by two factors: (1) by a discontinuance of the production of the specific hormone, and (2) by processes of

cell differentiations which accompany proliferative activity in the mammary gland and which lead to limitation of further growth activities with the setting up in their place of certain functional phenomena, as, for instance, secretion. We find the same phenomena in growth of glandular structures in general. Growth stimuli, of embryonal or regenerative character, induce cells which are as yet less differentiated to proliferate. This growth is accompanied by differentiation which itself limits growth and prepares the cells for secretory activity. While thus in the mammary gland certain secretory processes may be observed even while the corpus luteum is continuing to give off the specific growth substance (namely, as soon as certain changes in the character of the duct cells have taken place, which limit their proliferative potentialities and perhaps go so far as to enable them to secrete), still the active secretion takes place only after a large mass of tissue has been built up which has undergone this differentiating growth and then suddenly experiences a lack in the supply of the growth substance.

On the other hand, this latter phenomenon itself is not sufficient to induce a very active secretion if the preceding growth has not been sufficiently active. Thus we may find a somewhat rudimentary secretion in the newborn animal or child, when the growth substance given off by the mother ceases to act on the gland of the child; or even in the course of the estrous cycle some slight secretory activity has apparently been observed as soon as the growth stimulus of the follicular or lutein phase has ceased to act.

There is a further factor of importance in the secretory activity of the mammary gland. This is the process of suckling. As we have seen, secretion may begin in the sufficiently differentiating mammary gland, without suckling, at a time when the young are still in the uterus. Suckling, therefore, does not initiate secretion, but it makes possible the long-continued activity of the mammary gland and may augment the amount of secretion. Here again two factors probably come into play: (1) Without the emptying of the alveoli through suckling, the secreted material exerts an injurious pressure upon the secreting cells and instead of a secretion a degeneration and retrogression set in. (2) There is some evidence that in addition the process of suckling may stimulate the secretory activity. Thus it is stated that in cows the process of milking increases the amount of milk obtained. It is conceivable that through suckling a reflex stimulation, perhaps mediated through activation of the circulation, takes place. We know that suckling is able to set up reflexes, as for instance, those which promote the retrogression of the uterus during labor. This reflex action, if it should take place, would be strictly localized to the particular gland from which the milk is being withdrawn. It has no effect on the neighboring glands.

In consequence of the action of all these factors we find a fully developed secretion of milk only after labor, after suckling has been carried on

for some time, although in some animals, as in the goat, milk production may start in the latter part of pregnancy. In such cases the corpus luteum begins perhaps to degenerate some time before the completion of pregnancy. Preceding this full activity there may be a period in which colostrum is secreted. In accordance with the suggestion of Czerny (1890) it is usually assumed that colostrum is merely milk which has been changed through stagnation in the alveoli and the duct system either before suckling has been established or after suckling has ceased. However, it is possible that it is due to a secretory process as yet incomplete, or to one which is declining, owing to lack of suckling after a period of full activity.

There is exemplified in the secretion of the mammary gland a certain antagonism between mitotic cell proliferation and function to which, in a general way, Prenant seems first to have called attention. This antagonism is due mainly to a differentiation which takes place in the cell in the course of time and which is prevented or delayed by the effect of growth stimuli. The antagonism is reciprocal. Growth inhibits these alterations in the cells which tend to initiate certain functional activities, like secretion, and progressing differentiation is unfavorable to the development of those changes in the cell on which mitotic division depends. However, this antagonism is not absolute. We may still find mitoses in cells in which the presence of fat droplets indicates some secretion, and especially soon after labor we may find in the mammary gland mitotic proliferation due perhaps to the influence of the follicular hormone; but as the secretion becomes more and more fully established mitoses are rarer or are lacking altogether; there may occur instead, at least in certain species, amitotic division, as we shall discuss presently.

The antagonism between secretion and mitotic proliferation in the mammary gland is indicated also by certain other facts. Thus Kuramitsu and the writer (1921*a*, *b*) observed that in lactating guinea pigs maturation of follicles and ovulation are not suspended; but under these conditions the follicular phase of the sexual cycle does not seem to cause proliferation in the mammary gland; secretion continues. A new pregnancy beginning soon after labor does not prevent the active continuation of secretion. Apparently the proliferative stimuli reaching the actively secreting gland are unable to initiate definite growth processes. It would, however, be of interest to determine quantitatively whether after all the growth substances thus given off may not affect the condition of the gland to a certain extent. Conversely, it is possible that the deficient secretion during the later periods of pregnancy, notwithstanding far-advanced development of the alveoli, is due to a certain inhibiting effect on the gland cells of the growth stimuli emanating from the corpus luteum.

If we now consider the structural conditions underlying milk secretion there is considerable divergence of opinion among investigators, especially

as to the interpretation of the changes which can be observed in the alveoli. However, some points seem to be well established.

a. Variation in the height of the cells. While during pregnancy and especially at the end of pregnancy the character of the cells and the structure of the gland as a whole are found relatively homogeneous, great variations are seen during the process of secretion, as soon as suckling is regularly established. In some alveoli the cells are flat; in others, they are high; also in the same alveolus, cells may differ in size. According to Maeder (1922), the cells are higher in the peripheral alveoli of the rat; there is here less secretion and the nuclei are smaller. Also in the thyroid gland one can discover differences in the structure and apparently in the function of the peripheral and central acini. We suggest that these variations may be due to differences in the circulation in the peripheral and central parts of the gland. In the mammary gland, especially in the high cells, the fat is accumulated in the form of drops of different sizes. In addition, in such cells many granules are present, some of which may represent very small particles of fat. The fat droplets are situated especially in that part of the cell which protrudes like a pyramid or cone into the lumen of the alveolus. When the greater part of the fat has been discharged into the lumen the cells become lower and their inner outline separating them from the lumen of the alveolus is apparently indistinct for a time; subsequently a more sharply defined cell membrane seems to be reconstituted. Some authors assume also that the layer of aveolar cells as a whole becomes syncytial at this stage. However, it seems that with favorable methods of fixation, distinct outlines can be recognized at all times between the adjoining gland cells.

b. As to the nuclei, they are in general large, round or ovoid in shape. In the secreting glands the chromatin is distributed in clumps around the nuclear membrane, while in the proliferating gland of pregnancy, the chromatin particles are found distributed throughout the whole of the nucleus.

c. A further very interesting change which is observed in the lactating gland is the frequent occurrence of amitoses in the nuclei and the subsequent appearance of two or even more nuclei in a cell. However, amitoses are not equally frequent in all species of animals. They are quite common in the guinea pig and they occur in the rabbit, cow, dog and presumably in many other species, but they are rare, or may perhaps be entirely lacking, in the rat. This occurrence of amitoses does not imply that mitotic divisions do not take place at all; on the contrary, mitoses seem to occur regularly in the beginning of the secretory process soon after labor, and they may be found occasionally throughout the period of lactation in rat as well as guinea pig. Some nuclei undergo degenerative changes in the cell, namely, pycnosis, caryorrhexis, and caryolysis and these products of degeneration may apparently get into the lumen of the alveolus in this condition; especially the nuclei reaching into the papillary prominences of the cell seem to undergo these occasional degenerations. Some authors have described also the appearance of fat within the nuclei, a condition followed by the destruction of the nucleus.

d. In addition to these nuclear alterations various cytoplasmic structures have been connected with the process of fat production and secretion. Steinhaus (1892) described the occurrence of Altmann (fuchsinophile) granules in the lactating gland; gradually, and associated with the progressing synthesis of fat, these granules enlarge and change into threads or spirillae-like structures. This author, as well as Arnold (1905), attributes to such granules the function of producing fat out of glycerin and fatty acid. After the extrusion of the fat droplets and in the resting stage only a few granules remain. Limon has called attention to filaments in the basal part of the cell, thicker in the center and very fine at the ends, arranged around the nucleus. They are frequent in the actively secreting cell and fewer in the inactive cell. To these filaments also the function of syn-

thesizing fat has been attributed. In addition to these various structures mitochondria have been found in the secreting cells; however, according to Benoit (1922*a*, *b*) they seem to be not so numerous in the gland cells as in the surrounding fat cells of the stroma. This author observed, furthermore, in the mouse the development of fat within the mitochondrial structures in the fat cells directly adjoining the gland. He believes that the components of the fat molecule are supplied to the gland cell not through the capillaries, but directly from the adjoining fat cells. This conclusion he bases mainly on the fact that the fat tissue in the pregnant and lactating mouse consists of cells which are filled with small fat droplets suspended in the vacuoles of an areolar cytoplasm, while in the resting or retrogressing gland there occur instead typical fat cells which contain one large fat drop pressing on the nucleus. We may conclude, in accordance with what we found in other cases in regard to the relation between parenchyma and stroma, that stimuli reach not only the gland tissue proper, but also concurrently the surrounding stroma and here cause changes of a related nature. Thus the fat tissue during pregnancy and lactation may be considered to be in a state of stimulation, with an increased amount of protoplasm and with finely divided fat particles, while in the non-stimulated fat tissue the ordinary relation between fat and protoplasm exists. There is no direct indication that the fat tissue has the function of supplying the gland cells with the material necessary for the synthesis of fat.

1. *Mechanism of secretion in the mammary gland:*

As to the casein, some authors have assumed that its formation is due to a chemical combination of material derived from disintegrating nuclei and ordinary protein given off by the cells. However, it would be difficult to understand how a definite substance like casein could be produced in such a haphazard way, especially if we consider the irregularity of the disintegration of the nuclei. We must assume that in a similar way to fat it originates within the cell, and is subsequently secreted into the lumen of the alveolus. In general, three different views have been held as to the manner in which the cells give off the fat.

At the time when milk glands were assumed to correspond to sebaceous glands, it was held that the milk production like that of sebaceous material depended on the desquamation and degeneration of cells. Microscopic study of the structure of the mammary gland and its various modifications during secretion soon proved that this cannot be correct, although some gland cells do degenerate occasionally and are cast off into the lumen of the alveolus. Under certain pathological conditions such a process may, however, be quite pronounced (Guillebeau, 1916).

Heidenhain (1883) next interpreted the process of secretion as a cyclic one taking place between two successive periods of suckling. The actual secretion consists, according to this author, in a decapitation of the cell pyramid which protrudes into the lumen of the alveolus as soon as the main substances of which the milk consists have been produced within the cell. Thus protein, as well as fat droplets, is cast off from the cell and the secretion depends upon the partial destruction of the latter. In addition, certain nuclei disintegrate. This process is followed by a gradual recovery of the cell; it is at first low in height; a new inner cell membrane forms; again fat and presumably casein are produced, and with an increase in size a protrusion is made into the lumen in the typical manner. A number of investigators adopted this or a similar view.

On the other hand, a third group of authors, among whom Benda (1894) is especially prominent, believed that such a cell decapitation does not normally take place, but that

the fat droplets are pushed out from the intact gland cell into the lumen. In some cases cells have been seen in which apparently such a process was just taking place. Still other authors, like Brouha (1905*a*, *b*), assume that both processes, decapitation, as well as the pushing out of the fat droplets from the otherwise intact cell, may occur. Further comparative investigations of the mode of milk production in different species with the use of various kinds of fixatives are necessary to decide these problems. We may, however, conclude that, even if the fat droplets as such are pushed out of the cell, a process of secretion is involved different from the processes taking place in other glands, where we have presumably to deal with elimination of substances in a state of colloidal suspension or solution. The elimination of fat droplets—perhaps with some protoplasmic material adhering to some parts of the droplets—must necessarily mean a considerable interference with the normal activities of the cell.

As to the changes in the size of the gland cells which can be observed, some authors explain these as entirely due to mechanical factors, namely, the variable pressure of the secretion products on the wall of the alveoli. While this condition must play a part in determining the height of the cells, there can be little doubt that the preparation and accumulation of the material to be secreted are also factors which influence the size and shape of the gland cells.

An interesting problem is presented by the occurrence of nuclear amitoses in the secreting mammary gland cells, at least in a number of species of mammals. We have referred above to this process and have in particular pointed out the antagonism exemplified also in the case of the breast tissue between mitotic and amitotic division. We shall now discuss this problem still further from a more general point of view. If we consider the various conditions in which amitoses are found in mammalian tissues, we notice that we have to deal usually with cells in which a far-going differentiation in structure has taken place. Such a differentiation may be brought about, in some cases, through certain injurious conditions, such as obtain, for instance, in those cell rows of the regenerative epidermis which are further—but not too far—removed from the sources of oxygen.

A similar situation exists when in certain wandering cells and tumor cells the conditions for the supply of food in general, and of oxygen in particular, are to a certain extent interfered with. But the differentiation of tissues as such may, in other cases, even without a special curtailment of oxygen or foodstuffs, lead to such a change in the cell structure that mitoses cannot well take place in the stimulated cell; instead amitoses occur, sometimes leading to giant cell formation; at the same time agglutination between cells and development of syncytia formation may be favored. We find such conditions in malignant tumors, but also elsewhere. In some cases there may be a special stimulus which spurs on such cells to proliferate; but in other cases the usual metabolic environmental stimuli, which would not ordinarily cause proliferation, do have this effect in cells which through their structure are sensitized to stimuli of various kinds. In a strict sense we cannot speak, as Virchow did, of functional and formative stimuli, but only of functional and formative types of reaction. The

stimulus is not the specific factor, but the structure and accompanying specific metabolism of the cell are the specific factors which determine the response. In this sense we find in the lactating mammary gland a combination of the functional and formative types of reaction.

Yet in general, function is, to a certain extent, antagonistic to proliferation; but it is not function as such which is antagonistic, it is the cell differentiation underlying it; and there is, furthermore, a graded interference of this differentiation with cell or nuclear proliferation. At first the normal course of mitosis becomes difficult, although abnormal mitoses may still occur; then mitoses are replaced almost altogether by nuclear amitoses and occasional giant cell formations. Certain restricted or more pronounced liquefaction processes at the cell periphery may lead to agglutination and syncytial formation. These general theoretical considerations, which in principle the writer has expressed on various former occasions and which Barta (1926) has more recently applied to certain special conditions prevailing in tissue cultures of lymph glands, hold for the mammary gland as well. Here also the antagonism exists between mitoses and amitoses (Kuramitsu and Loeb, 1921*b*). Soon after labor a number of mitoses still occur; later they become rare, amitoses take their place and even the occurrence of pregnancy during lactation cannot induce the lactating gland to proliferate actively by mitoses. The pronounced development of gland tissue followed by the cessation of the growth stimuli emanating from the corpus luteum leads to the sensitization of the gland cells which makes the latter ready to respond to metabolic stimuli at the same time with secretory (functional) and proliferative reactions. The alternating filling of the alveoli with secretion, the resulting pressure on the capillaries, the emptying through suckling and the subsequent relief from this pressure and the interference with the cell life through expulsion of fat droplets, possibly with the loss of additional cell substance, bring about a complex of changes which are responsible for the process of milk production which is inserted between the stage of proliferation and degeneration of the tissue, and which corresponds perhaps to a very mild degree of tissue injury leading to reactions which represent a combination of a peculiar secretion and abnormal proliferative processes. The further detailed analysis of this complex remains still to be made; in particular, it will be necessary to determine how far the metabolic conditions as such act as a proliferative stimulus and how far the expulsion of material from the cell is responsible for these proliferative processes.

During the stage of colostrum formation, which occurs only in some species, especially in woman, but not in certain others, as for instance, the rat, characteristic cells are found filled with fat droplets and possessing one or several nuclei; sometimes, if degenerative processes have set in, the nucleus may apparently be lost entirely. There has been much discus-

sion as to the origin of these cells. They have been taken to be of epithelial origin, polynuclear leucocytes, lymphocytes and recently Gruber (1924) has suggested that they belong to the class of cells designated as histiocytes, monocytes or mononuclear leucocytes. The latter interpretation seems to be the most probable one. They take up fat particles by phagocytosis and carry them to lymph glands, spleen and liver.

We have previously seen that removal of the function of the corpus luteum is one of the most important factors in initiating lactation; extirpation of the ovaries, containing the functioning corpora lutea, during the second half of pregnancy at a time when the gland tissue has sufficiently developed, also has the effect of inducing milk production. On the other hand, the process of lactation has conversely some effects on the ovaries and also on the uterus. In some species, ovulation and even the full development of the follicles are suspended during lactation, but this statement does not apply generally. In the guinea pig maturation of follicles and ovulation occur normally in the lactating animal. Nevertheless, lactation increases retrogression of the uterus even more than does castration. We conclude, therefore, that in accordance with our previous interpretation, the influence of lactation on the uterus is a direct and not, as Hammond assumes, an indirect one, mediated through the ovaries, which are in the guinea pig actively functioning during lactation. In favor of our interpretation is also the fact that there is a direct reflex connection between the act of suckling and the uterus, the former eliciting uterine contraction.

As to the effect of castration on the gland in which lactation has already been initiated, it is generally assumed that it tends to prolong the activity of the mammary gland and also to increase the amount of certain constituents of milk. On the other hand, castration in the lactating guinea pig or rat does not influence the structural characteristics of milk production, in particular is the amitotic nuclear division not affected by this interference. We may, therefore, conclude that amitoses and also the isolated mitoses which occur in the mammary gland during lactation do not depend upon stimuli originating in the ovary, but that they are due to conditions intrinsic in the gland cells during the process of lactation (Kuramitsu and Loeb, 1921*b*).

2. *Retrogression of the mammary gland:*

As we have already stated, in the cycle of the mammary gland the stage of lactation is interpolated between the stage of proliferation and retrogression. As a general rule, cessation or diminution in the growth energy favors either differentiation and specific functioning of tissues, in case the preceding growth should have been orderly; or, it leads to the death of tissues and autolysis, in case the preceding growth has not been such that a well-arranged tissue has been formed; that is, one which is satisfactorily

supplied by blood vessels, and one in which the relation between blood vessels and tissues is so balanced that the supply of oxygen and foodstuffs cannot decrease below a certain level. Growth of tissues automatically provides the mechanisms which lead to a supply of blood vessels, and with this supply oxygen and foodstuffs are provided for the growing tissues. With the cessation of growth the specific stimuli acting on the vascular system of the growing tissue cease, also, and retrogressive changes take place. If this decrease in circulatory activity is only slight and if new circulatory stimuli are supplied by functional processes setting in after cessation of growth, and, in particular, in the case of the mammary gland, perhaps by the process of suckling, then the retrogression may be temporarily checked. However, with the cessation of suckling, the actual retrogression can no longer be delayed. Now the secreted material stagnates in the lumina of the alveoli and in the ducts and causes long-continued distention. The alveoli remain in a state in which they constantly press on each other, and this interferes with the circulation. The degree of interference must be unequally marked in different places. In the direct neighborhood of stronger vessels, the circulation may still be relatively satisfactory, while the tissue adjoining easily compressed capillaries must suffer more intensely.

Thus we find two processes going on hand in hand; viz., (1) atrophy of the tissue still fairly well supplied with oxygen and foodstuffs, and (2) actual degeneration, pycnosis, caryorrhexis, caryolysis of the nuclei, disintegration and solution processes of the cytoplasm, where the interference with the circulation is more pronounced. There is a possibility that, in addition to the diminution in circulation due to weaning, the tissues may be acted on unfavorably by substances produced from retained milk. However, this latter chemical effect is not necessary for the explanation of the retrogressive changes. We find comparable processes of a degenerative character in the corpus luteum and especially in experimental placentomata; also in the growth of malignant tumors similar degenerative and autolytic processes occur. Here they result solely from interference with the circulation. As soon as the growth stimuli cease, or as soon as parts of the tissue are no longer supplied with oxygen, owing to excessive, poorly coordinated growth, degeneration and autolysis set in. In certain respects we may analogize, in the last-named structures, the period of differentiation which precedes degeneration and which may also be the period of full function, with the period of lactation in the mammary gland.

Almost twenty-five years ago Hildebrandt (1904) explained lactation as due to an autolytic process within the cells. He believed that growth stimuli originating in the living and developing embryo prevented these autolytic processes. There is some truth underlying this idea; however, it is not so much the period of lactation as the period of retrogression in which autolytic processes play a prominent rôle. A reversibility of the processes of

differentiation, even if it should be possible given favorable conditions, does not actually occur in the mammary gland. The alveoli which have produced milk degenerate, as a rule, although a certain number of them may, in some cases, survive for a long time in a reduced condition. These are presumably structures which are relatively favorably situated in regard to oxygen supply; but a change of alveoli back into ducts from which they originated does not take place. On the contrary, not only alveoli undergo the degenerative changes of retrogression, but also the terminal parts of the ducts. A reduction in the ramification of the ducts may proceed very far; these may also become narrow and lose their lumen.

While the most pronounced retrogression is found after the period of pregnancy and lactation, some slight retrogressive changes occur also under conditions in which a less marked proliferation has taken place, followed by a sudden cessation of the growth stimuli; as, for instance, retrogression subsequent to growth of the breast in the newborn or subsequent to growth in the ordinary sexual cycle. In the latter condition, especially, the retrogressive changes may be slight and not be sufficient to remove entirely the material added through the preceding period of proliferaton. Thus, on the whole, the gland may become somewhat larger step by step. The slight retrogression which follows the follicular phase of growth in the ordinary estrous cycle is not usually preceded by secretory processes.

While in principle retrogressive changes are similar in all the species which have been carefully examined so far, there are some minor differences as to the rapidity of change and certain other features in different mammals.

Thus Kuramitsu and the writer (1921*b*) found that, in the rat, retrogression is much more rapid than in the guinea pig. In the rat, we, as well as Myers and Myers (1921), found that retrogression has progressed very far after eight or nine days. The period of complete retrogression was, however, reached somewhat later in our observations. According to Hammond (1925), retrogression may be rapid also in the rabbit. There does not seem to be a very marked difference between the retrogressive changes which follow the lactation period in the rat and guinea pig and those following labor in which the animals do not suckle their young. In both cases there may be at first some accumulation of milk in the ducts and in the lumina of the alveoli. Secretion does not cease at once; on the contrary, in the guinea pig, which does not nurse the young after labor, very slight secretion may proceed for some time here and there and occasional amitoses may still be found for several weeks; but in general, secretory activity ceases very soon in non-nursing animals and the stagnating fluid is gradually absorbed.

Mitoses begin to become more frequent pari passu with the decrease or cessation in amitoses. Thus in the guinea pig they are quite noticeable two and three weeks after the beginning of retrogression. These retrogressive changes lead to a diminution in size of the gland and to a disappearance of lobules and acini. In the guinea pig the morphological appearances of degeneration (caryorrhexis, caryolysis, vacuolization of cytoplasm) are more pronounced than in the rat. Connective tissue and later also fat tissue take the place of degenerated areas. There is not only a relative increase in connective tissue due to the shrinking of parenchyma, but also an actual proliferation of this tissue. At the same time an increase in wandering cells takes place in the stroma, which latter differ in

character in different species. In the guinea pig and rat, both lymphocytes and also polynuclear leucocytes increase; in woman the polynuclear leucocytes seem to be normally lacking and instead lymphocytes, plasma cells and some mast cells are found. We must assume that certain substances given off by the disintegrating gland tissue stimulate the surrounding connective tissue and attract the lymphocytes and some other wandering cells. The fibrous tissue thus produced exerts secondarily pressure on the glandular structures and in this way may contribute to their further destruction.

In general, processes of retrogression take a similar course during this period in the castrated and non-castrated guinea pig or rat. However, the number of mitoses and amitoses, as well as the number of wandering cells, seems to be smaller and the remaining gland cells lower in castrated animals. The effects of castration apparently superimpose themselves on the effect of lack of nursing.

The retrogression of the mammary gland is, however, not always complete; particularly in animals which have passed through a number of pregnancies, the acinar tissue does not often disappear entirely and thus variable amounts of gland tissue may remain. Incomplete retrogression may be associated with certain pathological phenomena; thus in women, especially, the connective tissue may remain hyperplastic and glandular structures may give origin to cystic formations; but we shall return later to the discussion of these changes.

We have seen that during lactation, as well as during the period of retrogression, endogenous stimuli are present in the mammary gland, which cause proliferative changes of a mitotic or an amitotic character. In addition hormonal influences, originating in the ovary, affect the mammary gland; although they are ineffective during the state of lactation, they become manifest again as soon as lactation has ceased.

VI. THE CHANGES IN THE STROMA OF THE MAMMARY GLAND

Before summarizing the changes which the connective tissue of the mammary gland undergoes accompanying the cyclic changes of the gland tissue proper, we must discuss very briefly some principles underlying connective tissue activity in general. In addition to the regenerative proliferation of connective tissue into defects, we may recognize certain other factors determining the activities of this tissue:

(1) Growth in response to contact substances. This factor can best be analyzed through transplantation of tissues. Here auto-, syngenesio- and homoio-substances given off by the transplanted parenchyma determine, in a quantitative manner, the intensity of growth of the connective tissue cells and the subsequent production, or lack of production, of fibrous tissue. At the same time these contact substances determine the growth of blood vessels. There is an interaction between the vessels and the connective tissue. How far the contact substances influence the growth of the connective tissue directly, how far the effect is an indirect one through the mediation of the vessels need further analysis. (2) Growth in response to hormone action. This we could demonstrate in the case of the growth of maternal placenta and placentomata, where it is specifically the connective tissue of the uterine mucosa which is sensitized by the corpus luteum to proliferative stimuli. The specificity of the connective tissue which is thus established depends presumably on contact substances given off

by the neighboring parenchyma. Thus we find that the connective tissue which, to a certain extent, has been kept in an active state—evidently through contact substances given off by the uterine epithelium and glandular structures—responds actively to the hormone. (3) Direction of the growth. This in the case of connective tissue as of other kinds of cells is largely determined through contact with solid substances. Such a reaction can best be analyzed through experimentally directed growth of connective tissue, in vivo or in vitro, in cotact with various coagula or solid substances. In particular, we found that the cell activities developing in experimental amebocyte tissue are favorable for this analysis.

These various factors we recognize also in the case of the stroma of the mammary gland. During fetal development the mammary gland grows from the epidermis downward into the underlying connective and fat tissue. Its stroma is, therefore, at first the ordinary subcutaneous tissue of the fetus. Gradually the gland tissue creates its own specific stroma. This must take place by means of contact substances given off by the gland cells; in an analogous way other specific tissue, like uterine epithelium, produces its own specific stroma through contact substances. It is soon possible to differentiate the stroma surrounding the terminal ducts, and in general the intralobular stroma from the stroma surrounding the excretory ducts. Around the latter the stroma is dense and fibrous, while around the terminal ducts and within the lobules it is more cellular. Here the fibroblasts are kept in an active state through the substances given off by the parenchyma, while around the larger ducts they enter the state of inactivity as shown by the formation of dense fibrous tissue, a condition which is somewhat akin to a degenerative change. We must assume—and this is a general condition—that the excretory ducts have a metabolism different from and less active than the more specialized epithelial structures to which they give origin.

In the mature gland we find a correspondence between the activity of the glandular elements and the surrounding connective tissue. Whenever, in the sexual cycle, there is proliferation in the epithelium, there is a corresponding activity, often expressed in mitoses, in the stroma. This statement applies to the intralobular stroma. Situated at a distance from the glandular elements, the interlobular and interlobar stroma, on the other hand, tends to be fibrous. Thus we find rhythmic changes during the estrous cycle as well as during pregnancy. Towards the end of pregnancy, with the slowing down of the proliferative processes in the epithelium, the connective tissue likewise becomes less active, while during the period of lactation the pressure exerted by the distended ducts and alveoli considerably decreases the amount of stroma in the gland and leads to the formation of only fine fibrillar septa. Then again during the retrogression following pregnancy, the connective tissue proliferates and forms dense fibrous tissue where glandular tissue had formerly been. Evidently the substances given off by the disintegrating parenchyma and its secretion products stimulate the stroma temporarily without, however, being able to maintain this

active proliferative condition and to prevent the formation of fibrous tissue. These substances of disintegration act in a way similar to homoio-substances.

During the menstrual cycle in woman the changes in the stroma are very pronounced. The stroma is finely fibrillar, somewhat edematous, in the stage of premenstrual glandular activity, while in the postmenstruum it becomes densely fibrous. Corresponding to the cyclic changes in the connective tissue, we find a certain cycle in the fat tissue in the mouse. During the resting stage, and during involution, the fat is deposited in the fat cells in the form of large vacuoles, while during pregnancy and lactation the fat tissue is finely vacuolar (Benoit, 1922*a*, *b*). How far all these cyclic changes are due to the hormonal influences which affect primarily the gland tissue, in addition to the contact substances originating in the latter and acting on the stroma, is difficult to determine at the present time; this remains a problem for further analysis.

After the involution period following lactation has ended, absorption processes may take place in the fibrous tissue, partly replacing it by fat tissue. In old age we find the stroma fibrous, containing relatively large amounts of elastic tissue and often undergoing degenerative processes. Here also the surrounding fat tissue may gradually be substituted for part of the fibrous stroma.

The stroma is infiltrated with various kinds of leucocytes, if we use the latter term in a general sense. To some extent they are specific for different species. Thus in adult woman and in the rat mast cells are found in the stroma; in woman plasma cells are also found; in the guinea pig eosinophile leucocytes seem to take the place of mast cells; but in the interlobular tissue eosinophiles are also in evidence in the lactating rat (Weatherford and Emmel, 1924). In all kinds of animals thus far examined lymphocytes are found in the stroma, especially during pregnancy, during the periods of colostrum formation, during the retrogression of the formerly active gland and they may still be visible in small collections around the vessels after the involution of the gland has been completed. They often penetrate actively between the gland cells into the lumen of the alveoli. The factors responsible for the appearance of these leucocytes are not simple. Evidently, different kinds of substances may attract them during various stages of the life cycle of the mammary gland.

We may, then, conclude that, during resting and retrogressing conditions, the mammary gland behaves not unlike a homoio-transplant, inasmuch as substances are given off by the gland cells which at first stimulate the fibroblasts, but fail to preserve them in an intact and active state and to prevent the formation of dense fibrous tissues on their part. The large ducts are in a similar resting state throughout and therefore they are always surrounded by relatively dense fibrous tissue. Proliferative states, and

quantitatively graded activity in general in the gland proper, are associated with a corresponding activity of connective tissue and fat tissue. How far these latter changes depend upon contact substances or on ovarian hormones, including those secreted by the corpus luteum, is as yet uncertain in most cases.

Collections of lymphocytes are, as in the case of homoio-transplantation, associated with the formation of intraalveolar fibrous tissue, but these cells are found also in large numbers during pregnancy and especially at the time of colostrum secretion. In amenorrhea, in stages of retrogression and in senescence, conditions observed in the stroma are very similar; all are characterized by the presence of a dense fibrous tissue and lymphocytic infiltration.

VII. THE MAMMARY GLAND UNDER EXPERIMENTAL AND PATHOLOGICAL CONDITIONS

We have concluded our analysis of the characteristics and modes of reaction of the cellular constituents of the mammary gland tissue under normal conditions. There are, however, certain experimentally produced states or pathological conditions which throw additional light on certain properties of these cells, which, while not specific for the mammary gland tissue, are of a fundamental character and are not apparent under ordinary conditions. These we shall enumerate briefly.

In the first place, tissue culture experiments with mammary gland (Maximow, 1925) indicate that a summation of growth stimuli may here be accomplished. The growth is found to be more active in vitro, in case the tissue of a pregnant animal is used than that induced in the resting or lactating gland. We have here evidently to deal with a summation of two proliferative reactions, namely, that caused by hormone and that caused by mechanical stimulation. Another instance of such summations of growth stimuli, and an especially striking one, is seen in the uterus, where a combination of hormone and mechanical action leads to the formation of placentomata.

These experiments in tissue culture further indicate that it is more particularly the less differentiated duct tissue rather than the more differentiated and functioning, lactating alveolar tissue which is responding to growth stimuli, although the acinar tissue may proliferate in some cases. Under conditions of in vitro growth the larger ducts can produce keratin and epithelial pearls not unlike those observed in squamous cell carcinoma, but to a limited extent also the terminal ducts or acinar tissue seem to be able to undergo a similar change. If we consider all the observations concerning the transformation of simple cylindrical and cuboidal epithelium into squamous epithelium, we may conclude that the ability to undergo such a transformation is widely spread, perhaps general, and that only the

threshold stimulation necessary for this transformation is different in different structures. The threshold is low, and therefore the changes are readily accomplished in excretory ducts, and it becomes successively higher the more we approach functioning terminal structures; but if adequate stimulation is used it can even be accomplished here in many cases. This transformation is, at the same time, an expression of increased proliferative activity.

Thus again is exemplified the progressive differentiation which takes place from the excretory duct in the direction of the terminal structures. This increasing differentiation implies, furthermore, a progressively changing metabolism, which is expressed in the variable tendency to produce keratin and to proliferate; it is expressed also in differences in the contact substances given off by the various structures as indicated by their effect on the underlying stroma. However, pearl formation, which is observed in invitro growth, does not in itself indicate a carcinomatous transformation of the tissue. If this were the case the tendency to pearl formation would have to be transmitted to successive generations of epithelial cells, even if they were placed under different conditions; and such a change in the character of the parenchyma has evidently not taken place. Indeed, similar pearl formation can be observed in normal skin epithelium growing in coagulated blood serum (Loeb). The stimulated epithelium of the mammary gland in in vitro experiments sends out pseudopods, shows ameboid movement and develops phagocytic activity like other epithelium similarly stimulated and freed from the restricting influences of the neighboring epithelium to which it is normally attached. Again, a like behavior has been noted in epidermis growing in coagulated blood serum. Stimulated under abnormal conditions the mammary gland tissue may divide by amitoses or undergo abnormal mitoses. It also shows the tendency to stereotropic reactions in its movements, as do all other tissues which become migratory. In contrast to the connective tissue the epithelium of the mammary gland has fibrinolytic power evidently of an enzymatic character. This power is, however, not, as is often assumed, a characteristic of epithelium as such in contradistinction to connective tissue, but it varies greatly in different kinds of tissue and in the corresponding tissues of different species (Fleisher and Lieb, 1915). In common with all connective tissue, the fibroblasts of the mammary gland have the tendency toward a radiating centrifugal growth into culture media.

The change from normal growth of mammary gland to carcinomatous growth and the fact that it is possible to propagate such transformed cells indefinitely through homoio-transplantation prove the potential immortality of the mammary gland cell. Associated with the carcinomatous transformation is a change in cellular metabolism; the cell becomes enabled to produce under almost anaerobic conditions lactic acid at the expense of

glucose (Warburg, Posener and Negelein, 1924), and at the same time it shows an increased tendency to proliferation and in many cases also an increased ameboid activity. As to the cause of this transformation, we have previously shown that it depends upon the cooperation of two kinds of factors; namely, (1) internal, hereditary factors, and (2) environmental factors which promote growth. The latter may be hormones given off by the ovary, or they may be external stimuli. Both sets of factors act in a graded manner and can, to some extent, replace each other. Thus, through elimination of the hormonal growth stimuli, at certain periods in the life of the animal, accomplished by means of castration, we can show that the readiness with which a carcinomatous transformation takes place depends upon the length of time during which the growth stimuli have been allowed to act in animals in which the necessary quantity of the hereditary factors are present.

If the action of the growth stimuli takes place under special conditions, as, for instance, when the growth stimuli of pregnancy are superimposed upon the stage of retrogression in mice, the change of normal into carcinomatous mammary gland tissue seems to be especially readily accomplished; in such cases the transformation results presumably even if the hereditary factor is not present. Thus we would interpret the recent experiments of Bagg (1926) which show that in strains of mice which are prevented from suckling their young and in which new pregnancies follow directly after labor, carcinoma of the breast develops in a large number of individuals. However, we have demonstrated that this carcinomatous transformation of the breast in mice is merely decreased in frequency but not prevented by not allowing mice to breed. The growth stimuli developing in pregnancy are, therefore, not the only ones which are effective. External stimulations like those produced by the application of tar may also produce carcinoma of the mammary gland (Yamagiwa and Murayama, 1924).

There are certain additional facts which point to the importance of imperfect retrogression of the mammary gland as a cause of pathological alteration in this organ. This imperfect retrogression indirectly supplies the conditions under which abnormal growth stimuli become effective and prepare the gland for a carcinomatous proliferation. Thus in a rat we observed fibro-adenomatous tumors and cyst formation which depended, in all probability, upon delayed and abnormal retrogression of the mammary gland. We have furthermore observed that, in transplanting such benign tumors, certain of them may show an increased transplantability, thus gaining some of the characteristics of carcinoma, and that while a number of them can only be autotransplanted successfully, others may, to a limited extent, also be homoio-transplanted. This points to the conclusion that transitional conditions exist in the life of the mammary gland between the normal and the carcinomatous state.

In agreement with this conclusion is the frequency with which certain proliferative changes are associated with retrogressive alterations in the senile breast, and there is, furthermore, in accordance with it the relationship in the human breast between the so-called chronic cystic mastitis (senile parenchymatous hypertrophy of Bloodgood) which apparently in many cases depends upon faulty retrogression of the mammary gland and the development of carcinoma. Of great interest is the fact, which we have pointed out on previous occasions, that the mammary gland cells, as soon as they have undergone the definite carcinomatous transformation, are no longer accessible to normal hormone growth regulation. The responsiveness of these cells to the hormones emanating in the follicles and in the corpus luteum depends evidently upon a certain metabolism which exists in the normal cells and, as we found, also in certain adenomatous glands; but it is destroyed by the presence of those endogenous growth stimuli and the correspondingly altered metabolism which are characteristic of carcinomatous mammary gland cells.

We find thus that the analysis of pathological states of the mammary gland gives an insight into certain characteristics of the gland cells which a study restricted to normal cells would not have revealed.

VIII. BIBLIOGRAPHY

Allen, Edgar. 1923. Racial and familial cyclic inheritance, etc. *Am. J. Anat.*, **32**, 203

Czerny, A. 1890. Über das Colostrum. *Prag. med. Wochenschr.*, **15**, 401.

Allen, Edgar, and Doisy, E. A. 1923. An ovarian hormone. *J. Med. Assn.*, **81**, 819.

Allen, Edgar, Pratt, J. P., and Doisy, E. A. 1925. The ovarian follicular hormone. Its distribution in human genital tissues. *J. Am. Med. Assn.*, **85**, 399.

Ancel, P., and Bouin, P. 1909. Action du corps jaune vrai sur la glande mammaire. *Compt. rend Soc. Biol.*, **66**, 605.

— 1911. Sur les fonctions du corps jaune gestatif. II. Sur le déterminisme du développement de la glande mammaire au cours de la gestation. *J. de Physiol. et de Path. Gén.*, **13**, 31.

— 1912. Sur l'évolution de la glande mammaire pendant la gestation. Déterminisme de la phase glandulaire gravitique. *C. rend. Soc. de Biol.*, **72**, 129.

——— 1924. Sur le déterminisme des phénomènes utérins et du développement gravitique de la glande mammaire. *C. rend. Assoc. des Anatomistes*, 19me réunion.

Arnold, J. 1905. Die Morphologie der Milch und Colostrumsecretion. *Ziegler's Beiträge*, **38**, 421.

Athias, A. 1915. L'activité sécrétoire de la glande mammaire hyperplasiée. *C. rend. Soc. de Biol.*, **78**, 410.

——— 1916*a*. Étude histologique d'ovaires greffés sur des Cobayes mâles chatrés et enlevés au moment de l'établissement de la sécrétion lactée. *Ibid.*, **79**, 553.

——— 1916*b*. Sur le déterminisme de l'hyperplasie de la glande mammaire et de la sécretion lactée. *Ibid.*, **79**, 557.

Bagg, H. J. 1926. The rôle of the functional activity in the production of mammary carcinoma. *Am. Naturalist*, **60**, 234.

Barta, Edmund. 1926. Deficient oxydation as a cause of giant cell formation in tissue cultures of lymph nodes. *Arch. f. exp. Zellf.*, **2**, 6.

Benda, C. 1894. Das Verhaltnis der Milchdrüse zu den Hautdrüsen. *Dermatol. Zeits.*, **1**, 94.

Benoit, J. 1922*a*. Sur les modifications de structure et la signification fonctionelle des cellules lipogènes interacineuses de la glande mammaire de la souris blanche. *C. rend. Assoc. d. Anatomists*, 17, Gand.

——— 1922*b*. Sur la participation de cellules glandulaires lipopexiques interacineuses à l'élaboration du lait, chez la souris blanche. *C. rend. Soc. de Biol.*, **86**, 609.

Berka, F. 1911. Die Brustdrüse in verschiedenen Alterstufen und während der Schwangerschaft. *Frankf. Zeitschr. f. Pathol.*, **8**, 203.

Bertkau, F. 190–. Ein Beitrag zur Anatomie und Physiol. der Milchdrüse. *Anat. Anz.*, **30**, 161.

Biedl, A. 1922. *Innere Sekretion*. Ed. 4, Berlin und Wien: Urban and Schwarzenberg, **3**, 1.

Bizzozero, G., and Vassale, G. 188–. Ueber die Erzeugung und die physiologische Regeneration der Drüsenzellen bei den Säugerthieren. *Virchow's Archiv*, **110**, 155.

Bloodgood, J. C. 1921. The pathology of chronic cystic mastitis of the female breast. *Arch. Surg.*, **3**, 445.

Bresslau, Ernst. 1902. Beiträge zur Entwicklungegeschichte der Mammarorgane bei den Beutelthieren. *Zeitschr. f. Morph. u. Anthropol.*, **4**, 261.

Brouha. 1905*a*. Recherches sur les diverses phases du développement et de l'activité de la mamelle. *Arch. de Biol.*, **21**, 459.

——— 1905*b*. Sur la signification morphologie de la mamelle. *Anat. Anz.*, **27**, 311.

Coen, Edmondo. 1888. Beiträge zur normalen und pathologischen Histologie der Milchdrüse. *Ziegler's Beiträge*, **2**, 83.

Dieckmann, H. 1925. Über die Histologie der Brustdrüse bei gestörtem und ungestörtem Menstruationsablauf, *Virchow's Archiv*, **256**, 321.

Dietrich, A. 1926. Rückbildungsvorgänge, Fibromatose u. Krebs d. Brustdrüse. *Deut. Zeitschr. f. Chirurgie*, **195**, 145.

Eggeling, H. 1904. Über ein wichtiges Stadium in d. Entwicklung. d. menschlichen Milchdrüse. *Anat. Anz.*, **24**, 595.

Fleisher, M. S., and Loeb, Leo. 1915. On tissue fibrinolysins. *J. Biol. Chem.*, **21**, 477.

Frank, R. T. 1911. The fuction of the ovary. *Surg., Gyn. and Obstetrics*, **13**, 36.

——— 1912. Zur Frage der experimentellen Milchauslösung. *Arch. f. Gyn.*, **97**, 183.

——— 1922. The ovary and the endocrinologist. *J. Am. Med. Assn.*, **78**, 181.

——— 1924. Function of the ovary. *J. Obst. and Gynecol.*, **8**, 573.

Frank, R. T., and Gustavson, R. C. 1925. The female sex hormone and the gestational gland. *J. Am. Med. Assoc.*, **84**, 1715.

Frank, R. T., Kingery, H. M., and Gustavson, R. C. 1925. The female sex hormone. II. An analysis of factors producing puberty. *Ibid.*, **85**, 1558.

Frank, R. T., and Unger, A. 1911. An experimental study of the causes which produce the growth of the mammary gland. *Arch. Int. Med.*, **7**, 812.

Gruber, G. B. 1924. Beiträge z. Histologie u. Pathologie d. Mamma. *Virchow's Archiv*, **248**, 397.

Guillebeau, Alfred. 1916. Die Neubildung von Drüsenzellen in d. Milchdrüse, etc. *Virchow's Archiv*, **221**, 1.

Halban, Josef. 1905. Die innere Secretion von Ovarium u. Placenta und ihre Bedeutung für d. Function d. Milchdrüse. *Arch. f. Gyn.*, **75**, 353.

Hammond, John. 1917. On the causes responsible for the developmental progress of the mammary glands in the rabbit during the latter part of pregnancy. *Proc. Roy. Soc.*, Ser. B., **89**, 534.

——— 1925. *Reproduction in the rabbit*. Edinburgh and London: Oliver and Boyd.

Hammond, J., and Marshall, F. H. A. 1914. The functional correlation between the ovaries, uterus and mammary glands in the rabbit, with observations on the oestrous cycle. *Proc. Roy. Soc.*, Ser. B., **87**, 422.

Hartmann, Carl. 1923. The oestrous cycle in the opossum. *Am. J. Anat.*, **32**, 353.

Heidenhain, R. 1883. Der Milchabsonderung. In Hermann's *Handbuch d. Physiologie*, **5**, 374.

Hildebrandt, P. 1904. Zur Lehre von der Milchbildung. Beiträge zur chemischen Physiologie und Pathologie. *Hofmeister's Beitr.*, **5**, 463.

Kahn, R. 1925. Die Innervation d. Milchdrüse. *Klin. Wochenschrift*, **4**, 2256.

Kehrer, F. A. 1901. Über gewisse synchrone Nervenerscheinungen und cyklische Vorgänge in den Genitalien u. anderen Organen. *Beiträge zur Geburtshilfe und Gynaecol.*, **4**, 228.

Knauer, Emil. 1900. Die Ovarientransplantation. *Arch. f. Gynaecol.*, **60**, 222.

Kuramitsu, Choizu, and Loeb, Leo. 1921*a*. The involution of the uterus following labor; the influence of castration and suckling on the process of involution. *Am. J. Physiol.*, **55**, 422.

——— 1921*b*. The effect of suckling and castration on the lactating mammary gland in rat and guinea-pig. *Ibid.*, **56**, 40.

Lacqueur, E., Hart, C. P., and DeTongh, S. E. 1926. Über das weibl. Sexualhormon, etc. *Deut. med. Wochenschr.*, **52**, 1247.

Lane, Claypon, J. E., and Starling, E. H. 1906. An experimental enquiry into the factors which determine the growth and activity of the mammary glands. *Proc. Royal Soc.*, Ser. B., **77**, 505.

Lathrop, A. E. C., and Loeb, Leo. 1916. On the part played by internal secretion in the spontaneous development of tumors. *J. Cancer Res.*, **1**, 1.

Limon, M. 1902. Phénomènes histologiques de la sécrétion lactée. *J. d. l'anat. et de la physiol.*, **38**, 14.

Lipschütz, A., and Adamberg, L. 1925. Hyperféminisation et rut prolongé, etc. *C. rend. Soc. de Biol.*, **93**, 1413.

Lipschütz, A., Tiitso, M., Svikul, D., and Versnyakov, S. 1926. Störung d. Spermatogenese u. weiblicher hormonaler Effekt. *Pfluger's Archiv*, **211**, 279.

Lipschütz, A., Tiitso, M., Voss, H. E. V., Vesnyakov, S., and Adamberg, L. 1926. Die geschlechtsverschiedene Reaktion auf d. ovarielle Transplantat. *Ibid.*, **211**, 697.

Lipschütz, A., and Voss, H. E. N. 1924. À propos du méchanisme de l'action inhibitrice du testicle dans l'hemaprohditisme expérimental glandulaire. *C. rend. Soc. Biol.*, **90**, 1332.

Loeb, Leo. 1909. The experimental production of the maternal placenta and the function of the corpus luteum. *J. Am. Med. Assoc.*, **53**, 1471.

——— 1911*a*. Uber die Bedeutung des Corpus luteum für die Periodizität des sexuellen Zyklus beim weiblichen Säugethierorganismus. *Deutsch. med. Wchnschr.*, **37**, 17.

——— 1911*b*. Der normale und pathologische Zyklus in Ovarium des Säugetiers. *Virchow's Archiv*, **206**, 278.

——— 1911*c*. The cyclic changes in the ovary of the guinea-pig. *J. Morphol.*, **22**, 37.

——— 1914. The correlation between the cyclic changes in uterus and ovaries in the guinea-pig. *Biol. Bull.*, **27**, 1.

——— 1916. Observations on the mode of origin of the fibroadenoma of the mammary gland in the rat and on the delayed retrogression of the mammary gland after the period of lactation. *J. Cancer Res.*, **1**, 415.

——— 1917*a*. The relation of the ovary to the uterus and mammary gland, from the experimental aspect. *Transactions Am. Gyn. Soc.*, **42**, 172.

—— 1917*b*. The experimental production of hypotypical ovaries through underfeeding. A contribution to analysis of sterility. *Biol. Bull.*, **32**, 91.

—— 1918*a*. The relation between the interstitial gland of the testicle, seminiferous tubules and the secondary sexual characters. *Ibid.*, **34**, 33.

—— 1918*b*. Corpus luteum and the periodicity in the sexual cycle. *Science*, **48**, 273.

—— 1919. Internal secretion as a factor in the origin of tumors. *J. Med. Res.*, **40**, 477.

—— 1921. Transplantation and individuality. *Biol. Bull.*, **40**, 143.

—— 1923*a*. The mechanism of the sexual cycle with special reference to the corpus luteum. *Am. J. Anat.*, **32**, 305.

—— 1923*b*. The effect of extirpation of the uterus on the life and function of the corpus liteum in the guinea-pig. *Proc. Exp. Biol. and Med.*, **20**, 441.

—— 1924. Quantitative relations between the factors causing cancer and the rapidity and frequency of the resulting cancerous transformation. *J. Cancer Res.*, **8**, 274.

Loeb, Leo, and Hesselberg, Cora. 1917. The cyclic changes in the mammary gland under normal and pathological conditions. I. The changes in the non-pregnant guinea-pig. *J. Exper. Med.*, **25**, 285.

—— II. The changes in the pregnant guinea-pig, the effect of lutein injections, etc. *Ibid.*, **2**, 305.

Loeb, Leo, and Kuramitsu, Choizu. 1921. The influence of lactation on the sexual cycle in the rat and guinea-pig. *Am. J. Physiol.*, **55**, 443.

Long, J. A., and Evans, H. McL. 1922. The oestrous cycle in the rat and its associated phenomena. *Memoirs University of California*, **6**, 148 pp.

Maeder, L. N. A. 1922. Changes in the mammary gland of the Albino rat during lactation and involution. *Am. J. Anat.*, **31**, 1.

Marshall, F. H. A. 1922. *The physiology of reproduction*. Ed. 2, London.

Marshall, F. H. A., and Halnan, E. T. 1917. On the post-oestrous changes occurring in the generative organs and mammary glands of the non-pregnant dog. *Proc. Royal Soc.*, Ser. B, **89**, 546.

Marshall, F. H. A., and Jolly, W. S. 1906. The oestrous cycle in the dog. *Phil. Trans. Royal Soc.*, Ser. B, **198**, 99.

Maximow, A. 1925. Über krebsähnliche Verwandlung der Milchdrüse in Gewebs-Kulturen. *Virchow's Archiv*, **256**, 813.

Michaelis, L. 1898. Beitrage zur Kenntniss der Milchsecretion. *Arch. f. mikr. Anat.*, **51**, 711.

Myers, F. J., and Myers, J. A. 1921. Gross changes in mammary gland in female albino rat during the period of involution. *Proc. Am. Assoc. Anat., Anat. Record*, **21**, 74.

Myers, J. A. 1916. The growth and distribution of the milk ducts and the development of the nipple in the Albino rat from birth to ten weeks of age. *Am. J. Anat.*, **19**, 353.

—— 1917. Studies on the mammary gland. IV. A comparison of the developing mammary glands in male and female albino rats, etc. *Anat. Record*, **13**, 205.

—— 1919. The histology of the mammary gland in the male and female albino rat from birth to ten weeks of age. *Am. J. Anat.*, **25**, 395.

Nissen, Franz. 1886. Über d. Verhalten d. Kerne in den Milchdrüsenzellen bei den Absonderung. *Arch. f. mikr. Anat.*, **26**, 337.

O'Donoghue, Chas. H. 1912. The growth-changes in the mammary apparatus of Dasyurus and the relation of the corpora lutea thereto. *Quart. J. Micr. Sc.*, **57**, 187.

Oslund, R. M. 1926. Cytorchid testes and testicular hormone production. *Am. J. Physiol.*, **77**, 76.

Ottolenghi, D. 1901. Beiträge zur Histologie d. functionirenden Milchdrüse. *Arch. f. mikr. Anat.*, **58**, 581.

Papanicolaou, G. N. 1923. Oestrous in mammals from a comparative point of view. *Am. J. Anat.*, **32**, 285.

Plant, Rahel. 1923. Über der Einfluss des Uterus und des Ovarien auf die Entwicklung der Brustdrüse. *Zeit. f. Biol.*, **79**, 263.

Robertson, T. B. 1922. *The chemical basis of growth and senescence.* Philadelphia and London: J. B. Lippincott Co.

Rosenburg, A. 1922. Uber menstruelle, durch d. Corpus luteum bedingte Mammarveränderungen. *Frankf. Zeitr. f. Pathologie*, **27**, 466.

Schil, L. 1912. *Recherches sur la glande mammaire*, etc. Thèse. Lyon.

Steinhaus, Julius. 1892. Die morphologie der milchabsonderung. *Archiv. f. Physiol.*, Supplement band **54**.

Stockard, C. R. 1923. The general morphological and physiological importance of the oestrous problem. *Am. J. Anat.*, **32**, 277.

Stockard, C. R., and Papanicolaou, G. N. 1917. The existence of a typical oestrous cycle in the guinea-pigs, with a study of its histological and physiological changes. *Am. J. Anat.*, **22**, 225.

Sutter, Monroe. 1919. On the behavior of the mammary epith. cell towards vital dyes, etc. *Proc. Am. Assoc. Anatomists, Anat. Record*, **16**, 164.

——— 1921. Cyclic changes in the mammary gland of the rat associated with oestrous cycle. *Proc. Am. Assoc. Anatomists, Ibid.*, **21**, 59.

Szabó, J. 1896. D. Milchdrüse im Ruhezustand und während ihrer Thätigkeit. *Arch. f. Anat. u. Entw.*, 352.

Vintemberger, P. 1925. Action des injections de liquide folliculaire sur la glande mammaire. *Arch. de Biol.*, **35**, 125.

Warburg, Otto, Posener, Karl, and Negelein, Erwin. 1924. Über den Stoffwechsel der Carcinomzelle. *Biochem. Ztschr.*, **152**, 309.

Weatherford, H. L., and Emmel, V. E. 1924. The relation of leucocytes to mammary secretion. *Anat. Record*, **27**, 191.

Yamagiwa, K., and Murayama, K. 1924. The experimental production of mammary carcinoma in rabbits. *J. Cancer Res.*, **8**, 119.

Section XXXV

INTERSTITIAL CELLS OF THE TESTIS

CONTENTS

SECTION XXXV

	PAGE
I. General Characteristics	1211
II. Distribution within Animal Series	1212
In fishes — In amphibians — In reptiles — In birds — In mammals.	
III. Finer Structure	1215
1. Nucleus	1215
2. Idiosome and centriole	1215
3. Mitochondria	1215
4. Secretion-like material	1217
5. Lipoids	1219
6. Pigment	1220
7. Crystalloids	1221
8. Golgi or reticular apparatus	1222
IV. Origin	1222
V. Cytomorphosis	1223
VI. Seasonal Variations	1226
VII. Hibernation	1229
VIII. Captivity	1230
IX. In Certain Abnormalities	1230
Cryptorchidism — Eunuchoidism — Hermaphroditism — In various diseases.	
X. Experimental Modifications	1232
1. Vasectomy	1232
2. Partial castration	1233
3. Testicular grafts	1234
4. Displaced testis	1235
5. Temperature of environment	1235
6. Roentgen ray	1235
7. Radium	1236
8. Reaction to healing of testicular wounds	1236
9. Over-feeding	1236
10. Inanition	1237
11. Deficiency diets	1238
12. Drugs and gland extracts	1239
13. Abolition of nerve supply to testis	1241
14. Interference with blood supply to testis	1241
15. Cross-breeding	1241
XI. Functional Significance	1241
XII. Bibliography	1243

Section XXXV

INTERSTITIAL CELLS OF THE TESTIS

A. T. RASMUSSEN

THE first published account of the interstitial cells of the testis seems to have been by Leydig in 1850. In connection with a description of the mammalian testis, he states that in the intertubular spaces are constantly present masses of cells containing either colorless or yellowish fatty globules that are unchanged by acetic acid. These cells are, therefore, frequently spoken of as the cells of Leydig. However, practically all the important contributions have appeared since 1896. The literature up to that date may be obtained from Bardeleben (1897).

Interest in them was especially aroused by Bouin and Ancel who in 1903 designated them as the "interstitial gland of the testis." These investigators concluded from the available evidence that they are the elements in the testis that liberate hormones which are important factors in the development of the secondary sex characters and sexual instincts. The term "puberty gland," introduced by Steinach in 1911, has found favor among a certain group of writers, although it is considered an inappropriate name by others (see, for example, Sand, 1923. The question of terminology is particularly well discussed by Lipschütz, 1924, p. 454).

The proportions to which the literature on this subject has grown in these few years is evident from the more recent treatises such as "Endocrinology and Metabolism," New York and London, 1922; Biedl's "Innere Secretion," Berlin, Ed. 4, Vol. 3, 1922; and "The Internal Secretions of the Sex Glands," Cambridge and Baltimore, 1924, by Lipschütz. Stieve (1921*a*) lists about one thousand references in a review of the development, structure and significance of the interstitial cells.

I. GENERAL CHARACTERISTICS

Typical interstitial cells are comparatively large, being generally 10μ to 25μ in diameter in mammals and birds, and 50μ or more in amphibia, although in the pig they also attain this size. They are polygonal in shape and epithelioid in appearance. The abundant cytoplasm is highly granular and contains almost invariably conspicuous lipoid globules, especially in the peripheral part of the cell. With ordinary technique that does not preserve or stain this lipoid the periphery of the cell is, therefore, more or less vacuolated as compared with the denser cytoplasm nearer the center. Hence the terms ectoplasm and endoplasm are frequently applied to these two regions of the cytoplasm.

They contain in general a single nearly round vesicular nucleus. Occasionally it is slightly elongated or even kidney-shaped. The latter seems to be the rule in the opossum. Bi- and even multi-nucleated cells have been described.

In many respects, therefore, they resemble, morphologically, gland cells, as has been pointed out frequently and especially emphasized recently by Wagner (1925).

They may be found as isolated cells scattered about in the ordinary loose connective tissue or more often arranged in irregular groups and strands. If very abundant, they form solid masses filling virtually the entire intertubular space. Occasionally they may form fairly uniform "nests," as illustrated in Figure 575. The isolation of such groups from each other and from surrounding connective tissue by a nucleated connective tissue sheath as reported by Nussbaum (1880) and Beissner (1898) is not typical and probably represents hasty interpretations. Ganfini (1903) states that in the European marmot many of the cells are in masses which are surrounded by "endothelium."

The arrangement of the cells about the blood vessels so as to form a more or less complete wall has been described by Boll (1871), Mihalkowics (1873), Nussbaum (1880), Regaud (1900), Bouin and Ancel (1903), Popoff (1909) and Winiwarter (1912); but this is not generally obvious. Where the interstitial cells occupy all the intertubular space they naturally come in close contact with the vessels as illustrated in Figures 568 and 575, but this is secondary, because if the interstitial cells of the same animal decrease in size so that the intertubular tissue becomes more open and loose, as in Figures 567 and 571, no special relationship between the interstitial cells and vessels seems to exist.

No special relation to the tunica propria of the tubular wall is apparent. Some interstitial cells may be closely applied but this is incidental.

II. DISTRIBUTION WITHIN THE ANIMAL SERIES

The presence of interstitial cells has not been established below the vertebrates; but these cells have been found at some stage of development in the vast majority of vertebrates examined. However, much more careful and systematic work along this line will have to be done before the species in which they are absent can be enumerated with any degree of certainty. As will be brought out later, there may be periods of the year when the interstitial cells are so atrophic that a casual examination might lead to the conclusion that no interstitial cells are present, whereas at a different season of the year the same individual would have been found to possess an abundance of typical interstitial cells. The subject is of considerable importance in connection with any attempt to assign specific functions to these cells.

1. *In fishes:*

During the last five years gland-like cells, apparently similar to the interstitial cells of higher animals, have been found at certain seasons of the year in the testis of about a dozen species of fish by Courrier (1921 to 1922), Kolmer and Scheminzky (1922), Remotti (1923), van Oordt (1924, 1925) and probably others. Champy (1923*b*) found none in three species (*Tinca vulgaris*, *Phoxinus laevis* and *Cyprinodontes viripares*), and Kolmer and Scheminzky also state that none could be found in a number of species which they examined. There are some indications that in certain species of fish, at least, some of the secondary sex characters (e.g., nuptial coloration) are dependent upon the testis (Kopeć, 1918; Blacher, 1926) although the castration experiments of Kopeć were not very successful (van Oordt, 1925).

2. *In amphibians:*

Much more is known about the presence of interstitial cells in the testis of amphibians. They have been found in at least a dozen species of urodeles (Humphrey, 1921; Kolmer and Koppányi, 1923 and others), in six or seven species of frogs and in the toad (for literature, see Lipschütz, 1924). In the discussions carried on between Champy, Aron and others, Champy (1923*a*) insists that there are tritons with no tissue in the testis that can be called glandular and, on the other hand, there are tritons with abundant glandular interstitial cells but without distinguishing sex characteristics. In *Molge cristata* (a triton with prominent secondary sex characteristics in the spring during the breeding season) no typical interstitial cells could be found in the testis by Aron (1921*a*, *b*); but he found a reddish-yellow mass of fatty tissue near the hilus of the testis, which persists during the breeding season and then atrophies. In cases where he was successful in destroying this tissue, the comb on the back and special coloration assumed during the breeding season underwent rapid regression, regardless of the fact that spermatogenesis was undisturbed. A special mass of interstitial cells near the testis has also been described by Kolmer and Koppányi (1923) in *Pleurodeles waltli*. Evidently there is a possibility that tissue of the nature of interstitial cells may be found outside of the testis proper.

3. *In reptiles:*

Mazzetti (1911) says that the testis of snakes is extraordinarily rich in interstitial cells during winter sleep. Pellegrini (1925) notes their presence in the tortoise (*Testudo orbicularis*) and lizard (*Lacerta muralis*). Ganfini (1902), Franckenberger (1922) and Reiss (1923) also find them in various species of *Lacerta*.

4. *In birds:*

Considerable disagreement has existed as to the existence, extent and duration of interstitial cells of the testis even in the domestic fowl (for earlier literature, see Boring and Pearl, 1917). The principal reasons for this situation are the facts that there may be remains of early sex cords that become infiltrated with fat so that they have the general appearance of interstitial cells and that there are periods when the interstitial cells are atrophic, as shown by Watson (1919) in the greenfinch and by Benoit (1923) in other species. The cells may appear gland-like only during the breeding period. In the drake, according to Parhon and Parhon (1922), the interstitial cells become abundant only as spermatogenesis diminishes. An evaluation of the amount of interstitial tissue in birds is deceiving because of the enormous increase in the tubules that occurs. Benoit (1922*c*) finds that while the tubules increase 1000 times from two weeks of age to maturity, the "gland-like" intertubular tissue has increased only eighty-six times, and how much of

this "gland-like" tissue is degenerating sex cords and how much true interstitial cells is not clear.

The real nature of the cells in the testis of the fowl termed "interstitial cell" have been discussed a great deal. Goodale (1919) thought they were a variety of eosinophilic leucocyte. Nonidez (1920) first concluded that they are large wandering cells (derived from lymphocytes) which have stored up fat and lost their wandering character; however, he has now abandoned this view.

Boring and Morgan (1918) found in the interstitial tissue of the testis of the Sebright cock (a hen-feathered breed) islands of large, well-defined cells having essentially the same appearance as the so-called lutein cells of the ovary of the hen. The assumption was made that these "lutein cells" are responsible for the female appearance of the Sebright cock and that after castration the elimination of these cells allowed full development of the secondary male sex characters, as noted first by Morgan (1915 to 1916). Nonidez (1922) is convinced from a careful study of their origin that these islands are not lutein cells but arise during degeneration of the sex cords and early seminiferous tubules as a result of fatty infiltration of certain elements of the seminal epithelium. This has been confirmed by Benoit (1923c) and Fell (1923) on other breeds.

That these "lutein" cells have anything to do with the sex reversal that occurs after castration of hen-feathered cocks, is doubtful because Pease (1922) and later Nonidez (1924*a*) report that they are not regularly present in fully mature hen-feathered and may be present in normal male-feathered fowls. See also the recent experiments on cross transplantation by Roxas (1926), where this question is fully discussed.

Benoit (1922, 1923) recognizes two types of interstitial cells in birds: glandular and non-glandular or connective tissue cells. To what extent his glandular variety includes the so-called "lutein" cells or degenerated cells of sex cords is difficult to say.

It seems fairly clear now that interstitial cells of the usual type are present in many birds, although in the past the so-called "lutein" cells or fat-laden cells from degenerating sex cords have been mistaken for interstitial cells in the strict sense.

5. In mammals:

In mammals these cells are very constant constituents of the testis, although the absolute and relative bulk with reference to the germinal epithelium and body weight varies within wide limits. Lenninger (1923) made rough estimates on nine species and found that there may be great differences in the number and size of the interstitial cells, even within the same family. Stieve (1923*a*, *b*) has published additional data of similar character. From the careful volumetric determinations on the testes of ten different species of mammals by Bascom and Osterud (1925), it appears that the total intertubular tissue represents from 3 to 10 per cent of the parenchyma in the guinea pig and as much as 38 per cent in the pig. While these percentages deal with a mixture of ordinary connective tissue, vascular tissue and interstitial cells, the variability expressed is not exaggerated because in general the greater the intertubular space the richer the tissue is in interstitial cells. Slotopolsky and Schinz (1925*a*), with even greater detail, find that, in man, the interstitial cells constitute from 8 to 28 (average, 12) per cent and that the intertubular connective tissue represents from 13.5 to 30 (average, 22) per cent, leaving about 66 per cent of the testis as tubules.

The unsupported statement by Odiorne and Simmons (1904), and apparently accepted by Coley (1908), that the normal adult human testis contains no interstitial cells cannot be accepted in the light of the abundant work that has been done on human material.

Marked cyclic changes are well established in a large number of mammals that have definite breeding periods separated by a long period of sexual inactivity (p. 1226).

III. FINER STRUCTURE

1. *The nucleus:*

There is usually only a single nucleus present, but Reinke (1896), Lenhossék (189-), Bouin and Ancel (1903), Winiwarter (1912), Rasmussen (1917), Duesberg (1918), Morgenstern (1923) and others have described binucleated cells (Fig. 547). Winiwarter illustrates an interstitial cell from the human testis with four nuclei (Fig. 548). In the fully formed cell the nucleus is practically always nearly round, except in the opossum where most of the nuclei have a depression on one side so that they are slightly kidney-shaped (Fig. 553). In the younger stages of differentiation they may be elongated and approach connective tissue nuclei in structure. In size they vary from 5μ to 8μ in mammals to 15μ to 18μ in amphibians.

One and occasionally two or even three nucleoli are fairly distinct after most staining methods. The chromatin net is greatly attenuated and consists of fine strands and rather small granules arranged in the usual manner.

2. *Idiosome and centriole:*

An unusually large idiosome is present in the endoplasm near the nucleus. Figures 1, 2 and 9 show these structures as seen in human material. Radiations are lacking or very inconspicuous. Two centrioles are generally found in resting mononucleated cells. In binucleated cells both Winiwarter (1912) in the human and Duesberg (1918) in the opossum found four centrioles (Fig. 547). Winiwarter found as high as eight centrioles within a single cell (Fig. 548). In most animals they are round but in the human they are rod-shaped.

3. *Mitochondria:*

These bodies seem to have been first specifically mentioned in connection with the interstitial cells of the testis by Jordan (1911) in the opossum. Whitehead (1912*a*) about this time concluded that some of the granules he had previously described in the cat and pig were mitochondria. This same year Winiwarter (1912) published beautiful illustrations of their appearance in human material as reproduced here in Figures 550, 551 and 552. Rubaschkin (1912) evidently also recognized them in the guinea pig. Special attention was paid to the mitochondria in the interstitial cells of the testis by Duesberg (1918) in the opossum and by Rasmussen (1918) in the woodchuck. Most of the cytological studies since then have mentioned mitochondria where proper technique has been employed.

As shown in Figures 550, 551 and 552, in man they are in the form of both granules and filaments of various lengths. In the oppossum they are mostly short curved rods (Fig. 554), although filaments of various lengths

FIGS. 545–553.—Interstitial cells of human testes. Flemming's fixer. (After Winiwarter.)

FIG. 545.—From a twenty-five-year-old subject; 2 rod-like centrosomes, idiosome, round lipoid globules of various sizes, 4 large black crystalloids (cut obliquely and hence oblong in section), peripheral ectoplasm, central granular endoplasm, and accentrically placed nucleus. Triple stain.

FIG. 546.—Same material and technique as in Figure 545, 2 nucleoli, 4 centrosomes, lipoid.

FIG. 547.—Same material and technique as in Figure 545; 2 nuclei, 4 centrosomes and a large number of oval bodies ("corps riziformes") in left pole.

FIG. 548.—Same material and technique as in Figure 545. A rare cell with 4 nuclei and 8 centrosomes.

also occur. Only round granules were observed in the woodchuck (Rasmussen, 1918) (Figs. 572 to 577) and rooster (Massaglia, 1920). Humphrey (1921) in *Necturus*, Wagner (1925) in mouse, guinea pig and cat also illustrate only granules. In the rabbit Okuneff (1923) found mostly the granular type with a few short rods.

Mitochondria are most numerous in the central cytoplasm or perinuclear region. In the peripheral zone they are apt to be found in groups between the lipoid globules and other inclusions.

According to Duesberg (1918) the mitochondria of interstitial cells are especially labile. In Figures 555–558 this author illustrates several stages of what he considers to be artificially modified mitochondria and networks which have been formed from them by the action of the fixing agent. Figure 554 shows an early stage of such chondriolysis. Humphrey (1921) also noted peculiar networks in urodeles but they seemed to have arisen from fuchsinophile granules (see below) somewhat larger than mitochondria.

4. *Secretion-like material:*

Various round granules larger than mitochondria have been repeatedly described by various investigators. They are most numerous in the dense central cytoplasm, as illustrated in Figures 574a and 576b. They are well stained by methods that bring out zymogen granules and are difficult to separate from mitochondria with the aid of mitochondrial technique, although they are more easily fixed and stained than mitochondria. They usually become very prominent when the interstitial cells undergo hypertrophy. Humphrey (1921) concluded, upon the basis of staining reactions in urodeles, that the granules of this general description in the hypertrophied cell probably contain more lipoid than mitochondria; but in the woodchuck and many other animals there are no indications of much lipoid in their composition. Undoubtedly there is considerable difference in the composition of these secretion-like granules in different species and in the same species at different periods of their cytogenesis. The histological pictures

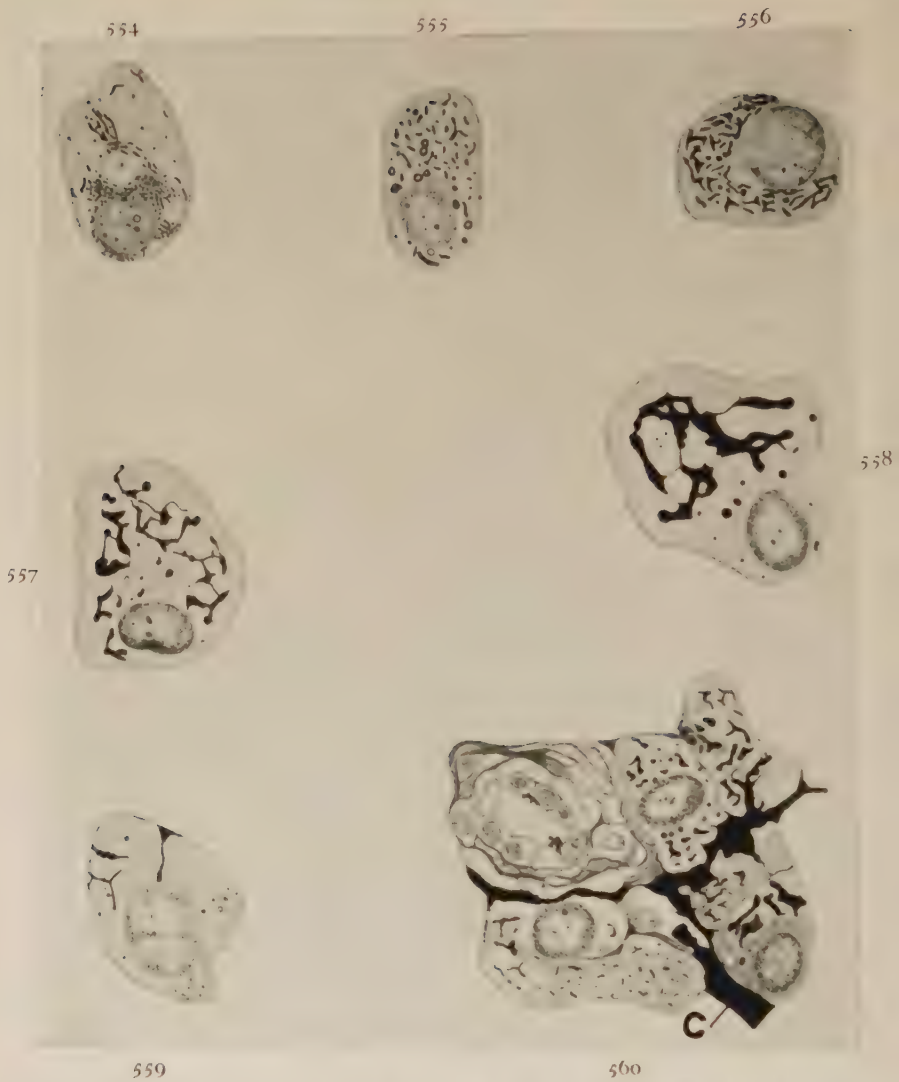
Fig. 549. From a forty-one-year-old subject. Same technique as in Figure 545. A group of cells containing characteristic large and small crystalloids in longitudinal view.

Fig. 550.—From a twenty-one-year-old subject. Mitochondria as granules and filament. Benda's mitochondrial stain.

Fig. 551.—Same material and technique as in Figure 550. Cell filled with short rod-like mitochondria ("chondriocontes").

Fig. 552.—From a 5 cm. human fetus showing the mitochondria. Heidenhain's iron hematoxylin and eosin.

Fig. 553.—Same material and technique as in Figure 552. Lipoids show as small gray granules, mitochondria as small black granules and short rods, idiosome and diplosome.



FIGS. 554–560.—Interstitial cells of the testis of the opossum (Didelphys.) Benda's mitochondrial technique. (After Duesberg.)

FIG. 554.—Normal mitochondria ("chondriosomes").

FIG. 555.—Early stage of mitochondrial modification thought to be brought about by the action of the reagents used in fixation.

FIGS. 556, 557 and 558 show different types of networks formed of modified mitochondria, according to Duesberg.

FIG. 559.—A cell with extracellular secretion-like material penetrating the cytoplasm, giving one the impression that there are intracellular canaliculi.

FIG. 560.—A group of interstitial cells with intracellular and intercellular accumulation of secretion-like products. The same material fills an adjacent capillary (c).

such as shown in Figures 5-73a, 5-74a, and 5-76b suggest that they might arise from mitochondria. It is also possible that they represent poorly preserved mitochondria since, as stated above, the mitochondria of interstitial cells seem to be especially prone to assume various enlarged forms in consequence of faulty fixation.

Whitehead (1904 to 1912), in several papers, mentions granules 1μ to 2μ in diameter each within a small vacuole and scattered through the cytoplasm especially at the periphery of the cell (Fig. 561). These are abundant in the cat, pig (normal and undescended testes) and man; fairly abundant in the dog and sheep; while in the rat, gray squirrel and rabbit only a few were found (Whitehead, 1908*b*). They may be stained with either basic (gentian violet) or acid (acid fuchsin) dyes, although they have a greater affinity for the latter.

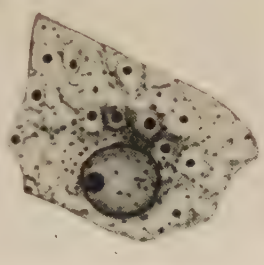


FIG. 561. —Interstitial cell from the testis of a cat. Formalin fixation. Iron hematoxylin. Illustrates large granules (not mitochondria) within vacuoles. Mitochondria are the smaller granules between the vacuoles. (After Whitehead.)

In the opossum Duesberg (1918) found in some of the cells granules which he considered secretion products. Granular or amorphous material of this nature he found in considerable quantity in the intercellular spaces and penetrating into the cytoplasm of adjacent cells along intracellular channels (Figs. 559 and 560). As shown in Figure 560, this same material may be found in neighboring blood vessels. The histological appearance strongly suggests that this is a secretory product finding its way into the circulation. Wagner (1923, 1925), in the guinea pig and rabbit, also finds more or less homogeneous material in the intercellular spaces, and in the cat and mouse he noted clear globules more or less in association with lipoid apparently being absorbed by blood vessels or lymph vessels.

Harms (1922) states that he has often seen histological pictures which indicate that the interstitial cells are discharging substances into the capillaries; but the material which they pass on to the blood stream, he thinks, has been formed by the epithelium of the tubules and has only been stored in the interstitial cells. For further discussion of this topic, see Wagner (1925).

5. *Lipoids:*

Abundant fatty globules in the cytoplasm are one of the principal characteristics of the fully developed interstitial cell. There are a few exceptions, notably the pig and opossum, in which these cells are almost free from discrete lipoid droplets. Plato (1896) states that in the wild boar where these cells are unusually prominent only a few fatty granules are

found. There may be considerable variation among the cells of the same animal especially in cases where there are successive periods of atrophy and hypertrophy.

The lipoid is usually described as being in the form of round, discrete globules fairly uniform in size, as shown in Figures 2–3a and 563, although in some cases (e.g., human) there may be considerable variation in their size (Figs. 545 and 546). Wagner (1925), however, presents evidence indicating that many seemingly solid globules of lipoid are in reality vacuoles filled with a non-lipoid substance with only a film or partial film of lipoid on the surface. He finds the existence of "sickle"-shaped masses of lipoid related to vacuoles, as shown in Figure 562, to be the general rule in such mammals as the mouse, guinea pig, rabbit and man. The writer noted this appearance occasionally in the woodchuck but interpreted it as being due to imperfect action of the mordant. In the pig such vacuoles free from lipoid are abundant (Whitehead).

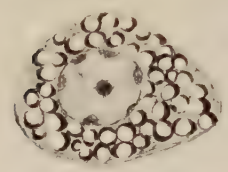


FIG. 562. — Interstitial cell from testis of adult mouse showing the lipoid as sickle-shaped bodies closely applied to vacuoles. Sudan III and hematoxylin. (After Wagner).

The microchemical studies of Ciaccio (1910) on amphibians and various mammals and especially of Whitehead (1912*b*) on the cat indicate that most of the fatty material, in the cat at least, is of phospholipin nature (phosphatides) with a small amount of cholesterol esters and probably of neutral fats. That there are also glycolipins is indicated by Sorg (1924), who reported cerebrosides as well as phosphatides in the interstitial cells of cattle; while Lotz and Jaffé (1924) mention the presence of cerebrosides in the interstitial cells of human adults who died of general affections but state that the amount of cholesterol esters and of cholesterol is greater than the amount of phosphatides and of cerebrosides.

The rôle of the lipoid is obscure. The view that it constitutes the material from which the hormones are formed was held by Loisel (1902), while Ganfini (1902) and some later writers, e.g., Pellegrini (1925), consider it to be the specific secretion. The effects of lipoid extracts of the testis such as reported by Iscovesco (1913) suggest strongly the presence of pharmacodynamic lipoids in the testis. On the other hand, the almost total absence of this substance in some species and the presence of other inclusions militate against this view.

6. *Pigment:*

The presence of brown pigment granules has been noted frequently in the interstitial cells of various animals, but it is absent in others. In the human it is usually not present until the adult stage is reached (Cordes, 1898). According to Sehrt (1904), the pigment is mostly lipochrome; but there may be some pigment present that is soluble in water.

Loisel (1904) speaks of two kinds of pigment in the rooster. One is clear and the other is dark and insoluble. The latter is found in the cells which he regards merely as hypertrophied connective tissue cells, while the clear pigment is found in what he considers true interstitial cells. In the horse Bouin and Ancel (1905*b*) found that there are large, discrete cells containing much pigment. The presence of certain cells with abundance of pigment among many with little or no pigment is illustrated probably best in the woodchuck or American marmot (Figs. 567 and 571). These highly pigmented cells are largest and most numerous in late summer at the close of interstitial cell atrophy and gradually diminish in size and number so that only a few remain by the following spring. As is shown in Figures 572P, 573P, 574P and 577P, the nucleus is usually irregular in outline and frequently crowded to the periphery of the cell. The round pigment granules are variable in size and virtually fill the entire cell. Some of them tend to clump together in morula-like masses in which the individual granules gradually coalesce with the formation of large, round pigment masses as illustrated in Figure 574P. In short, the indications are that these highly pigmented cells are degenerating interstitial cells. The pigment in the woodchuck is apparently derived from the fuchsinophile granules and possibly from part of the lipoid during the annual period of interstitial cell atrophy which occurs regularly in midsummer, as described on page 1226.

7. Crystalloids:

Reinke (1896) was the first to describe elongated crystalloids in human interstitial cells. He thought he saw them pass into the lymph vessels. The occurrence of these bodies has been abundantly verified in human material, but not their passage into the lymph stream. Figure 5 illustrates their appearance as seen in longitudinal section. In Figure 545 they are cut obliquely. Mathieu (1898) reported the presence of crystalloid in the cat but his observation seems to lack confirmation. Crystalloids have generally been regarded as characteristic of human interstitial cells; but Duesberg (1918) found them in many of the interstitial cells of the opossum. He describes two types. One of these is characterized by being thick and may exhibit a light central zone running lengthwise. One end may be split. In rare cases they are surrounded by a clear space. This is also the case in man. Generally only one crystalloid of this kind is found in a single cell in the opossum. The other somewhat rarer type is thin with pointed ends and is usually curved.

Lenhossék (1897) called certain smaller structures crystalloids but Duesberg thinks these correspond to the oval bodies or "corps riziformes" which Winiwarter (1912) says are always present in considerable quantity in the human testis. Sometimes they are distributed through the entire cell, or they may be confined to one pole, as represented in Figure 547. Winiwarter

regards these as transitory substances, quite different from Reinke's crystalloids, which are apparently composed of globulin. For further discussion and the literature, see Bukofzer (1924).

Albuminous crystals were found by Lenninger (1923) in the interstitial tissue of the deer just preceding rutting.

In the human testis the crystalloids do not appear until puberty but persist thereafter (Reinke, 1896; Lubarsch, 1896; Bardeleben, 1897), although Bouin and Ancel (1904*e*) and Bukofzer (1924) find that they decrease in old age. Bukofzer is inclined to the view that these crystalloids are associated in some way with somewhat similar structures found in the epithelium of the tubules (crystalloids of Spangaro and Lubarsch) and represent reserve material for the development of spermatozoa.

8. *Golgi or reticular apparatus:*

This component has been studied by Duesberg in the opossum. Figure 563 shows a typical arrangement of the apparatus concentrated near one

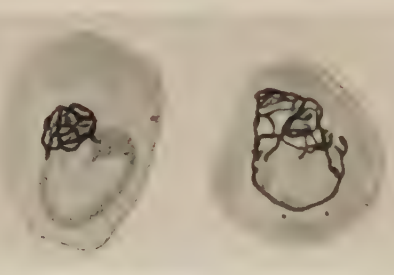


FIG. 563. FIG. 564.

FIGS. 563 and 564. - Interstitial cells from testis of opossum showing two forms of the apparatus of Golgi. Cajal's method. Counterstained with Ehrlich's in 563 and with methyl green in 564. (After Duesberg.)

pole of the nucleus. Occasionally cells are found in which the Golgi apparatus extends to the opposite pole, as shown in Figure 564.

IV. ORIGIN

There has been much interest taken in the embryological origin of the interstitial cells of the testis, partly because this might throw some light on their function. A few have assumed that if they should be glandular, an epithelial origin would be expected. The great bulk of the best embryological work indicates that they come from mesenchyme which is indistinguishable from that which gives origin to fibrous connective tissue: (Plato, 1897, in the cat; Maximow, 1899, in the dog and other animals; Ancel and Bouin, 1903, in the pig; Allen, 1904, in the pig and the rabbit; Whitehead, 1904, in the pig; Sainmont, 1906 to 1907, in the cat; Firket, 1920, in the fowl;

Kronfeld, 1921; Bascom, 1923, in cattle; Stieve, 1923*b*, in the mouse; van Oordt, 1924, in fish; and others).

Loisel (1904) in the chick, Rubaschkin (1912) in the guinea pig, Benoit (1923*c*) in the chick, Scheunig (1923) in the human, and a few others are inclined to accept an origin from the early sex cords. The dark pigmented cells in the fowl, according to Loisel, arise from connective tissue cells, but these are not regarded as true interstitial cells by this writer (p. 1221). Lubarsch (1921) and Battaglia (1925) consider that there are enlarged connective tissue cells filled with lipoid that resemble interstitial cells but which are in reality not true cells of Leydig. Battaglia supports his view by injecting tubercle bacilli into guinea pigs which causes connective tissue cells of the intertubular region and of the walls of the tubules to take on the general characteristics of true interstitial cells.

Bouin and Ancel (1905) concluded that in the horse they may also spring from lymphoid tissue located between the early seminal tubules. It may be recalled that Goodale (1919) thought that in the chick they differentiate from primitive blood cells. Champy (1913) in the case of the frog and Courrier (1922) in fish considered that they were derived from leucocytes. This was also the earlier views of Nonidez (1920) who thought wandering blood cells stored up fat, lost their wandering character and thus become interstitial cells. Later (1924*a*), in a re-study of this question, he finds that his earlier notions of a vascular origin are incorrect. Čejka (1923) gives a schema of histogenesis which shows their origin from primitive mesenchymal cells through lymphocytes and wandering cells to polyblasts of Maximov, which Čejka considers interstitial cells.

An old idea was that the interstitial cells spring from the epithelium of the Wolffian body; but Whitehead (1904) showed that this is impossible in the pig, for the interstitial cells appear before there is any connection between the testis and the Wolffian body.

Berger (1922) thinks that probably they should be regarded as paraganglia which implies a neural ectodermal origin, and Harvey (1875) considered them actual nerve cells.

In man the stage at which they are first recognizable is given as 45 mm. embryos by Felix (1912), while Kitahara (1923) found them well differentiated in 43 mm. fetuses.

V. CYTOMORPHOSIS

As shown on page 1222, the vast majority of investigators agree that the interstitial cells of the testis arise from cells which are indistinguishable from fibroblasts. Their scanty cytoplasm increases in amount and becomes more granular. Lipoid globules may appear early. Mitochondria increase and other secretion-like granules make their appearance. The nucleus rapidly becomes nearly round and lighter in staining reaction. The cytoplasm continues to increase. In most cases this is largely due to the accumulation of lipoid. Finally the adult stage is reached.

The particular characteristics of the interstitial cells of the rat at various ages was described as early as 1900 by Regaud. Maximow (1899) gave some data on the dog, but the most detailed description is by Whitehead (1904, 1905) in the pig. Whitehead found that there are two phases of growth separated by a wide interval of atrophy. The first period of accelerated growth is between the 2.4 cm. and the 3.5 cm. stages. During the atrophy which follows and continues to the 20 cm. stage, the cells are reduced almost

to naked nuclei. Meanwhile the tubules are growing rapidly. The second period of rapid growth commences at about the 20 cm. stage and attains a maximum near term (28 cm. stage). A second interval of gradual atrophy then sets in until about five months of age, at which time many cells are again reduced to little more than nuclei.

Aron (1921), also in the boar and Bouin and Ancel (1905*b*) in the horse describe two more or less distinct generations of cells—a fetal generation followed by one which appears with the onset of spermatogenesis. There are minor differences between the two. Smith (1919) finds in dogs a noticeable increase in interstitial cells and their lipoid content towards the adult stage with atrophy, loss of fat and increased pigmentation in old age; while Goodpasture (1918) seems to have found them to be more numerous in old dogs. Stieve (1923*b*) gives quantitative data on the amount of interstitial tissue in the mouse from birth to old age, which indicate an absolute increase with age in both number and size of the interstitial cells with possibly a slight decline in old age. Bascom (1923) finds them relatively less abundant in cattle near birth. By means of a method essentially the same as that used by Stieve, Riccardi (1924) gives some data from fetal and postfetal horses, cattle and dogs. The most accurate evaluation of the absolute and relative amount of interstitial cells is the work done by Lundgren (1925) on 59 rabbits from one month to thirty-four months of age, which shows an absolute increase to ten months and a relative increase (with reference to the testis as a whole) to five months (puberty). In both respects there is a distinct decrease to twelve months of age, after which there is a very slow decline to three years, the age of the oldest animal involved.

Data on the human also indicate the existence of considerable variability in the interstitial cells at different ages (Hofmeister, 18–2; Spangaro, 1901; Thaler, 1904; Branca and Basseta, 190–; Kasai, 1908; Mita, 1914; Mott, 1919; Ishibashi, 1919; Goddard, 1920; Benda, 1921; Diamantopoulos, 1921; Lahm, 1922; Scheunig, 1923; Kitahara, 1923; Oppermann and Jaffé, 1924). By about the middle of intrauterine life they constitute a considerable part of the testis. The individual cells are large. Then both nucleus and cytoplasm decrease somewhat in size as birth approaches, when they constitute a distinctly smaller proportion of the entire organ, but may be relatively rich in lipoid. After two years of age practically no lipoid in general is demonstrable until towards puberty when the germinal epithelium begins to mature. At puberty a marked increase in size and total bulk takes place and the cells become loaded with lipoid. Diamantopoulos (1921), from a study of ninety-nine pairs of testes from newborn infants to individuals eighteen years of age, believes that many under-developed testes are at this time brought up practically to normal. The impression is that there is a slight decrease in the interstitial cells during the next few years. This is probably only relative, owing to the growth of tubules. At about twenty-

one years distinct pigment granules appear in some of the cells. In old age there appears to be a relative increase in cells again, but this is mostly due to slow atrophy of the tubules. This is accompanied by increased pigmentation and loss of crystalloids (Bouin and Ancel, 1904*e*).

There is considerable individual variability, which explains much of the discrepancy in reports where only a few individuals are involved at any one stage of development. However, some differences of opinion cannot be attributed to this cause. Thus Lubarsch (1921) does not consider that there are any interstitial cells in the human testis until after puberty. The large cells, seen before this time, he regards merely as connective tissue cells.

The two types of cells shown by Wagner (1925) by intravital staining of adult mice with pyrrol blue (which causes the smaller cells to become blue while larger ones remain unstained, as shown in Figure 565) probably represent different stages of cytomorphosis. A similar differentiation is illustrated by Wagner (1925) in the mole by staining fixed tissue with acid fuchsin and thionin. The smaller cells stain red and the larger ones blue. However, observations on their reaction to vital stains are conflicting. Addison and Thorington (1917) conclude that when trypan blue is injected subcutaneously into white mice and white rats the interstitial cells of the testis are free from the dye. The coloring matter seemed to be taken up by elongated or more often by rounded or polyhedral cells that are regarded as clasmatocytes. Evans and Schulemann (1914), on the contrary, found that trypan blue stained the interstitial cells of the testis brilliantly.

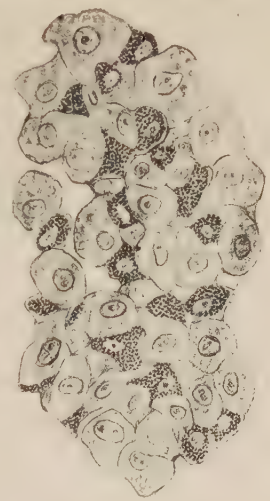


FIG. 565. Interstitial cells from adult mouse. Vital staining with pyrrol-blue by Goldmann's method for six weeks. Smaller cells become blue (here shown in black) while most of the cells remain relatively unstained. (After Wagner.)

A noticeable feature throughout the literature on both animals and man is the rarity with which mitotic figures are encountered in fully formed interstitial cells. A few instances in adults are on record, but the impression is quite general that when there is hyperplasia in later stages of development, the increase in number is brought about by enlargement of connective tissue cells or small cells indistinguishable from fibroblasts. This is illustrated by Figures 572, 573 and 574 which show the cytological changes that take place in the spring during the breeding period of the woodchuck. This phase is discussed in the next division.

Bardeleben (1897) did not see any mitotic figures in the interstitial cells of executed criminals, but frequently saw what appeared to be evidences of direct cell division. In the pig Ancel and Bouin (1903) claim that during

early stages they proliferate by amitosis. Stieve (1922) also gives illustrations of direct cell division in the mature gander. Kyrle (1911) discusses the possibility that the hyperplasia following roentgen-ray treatment in dogs is due to amitotic cell division. However, Maximow (1899) found abundant mitotic figures in large interstitial cells in the neighborhood of aseptic injuries of the testis in common laboratory mammals within twenty-four hours after the experimentally produced lesion. This indicates that they can divide under the proper stimulus.

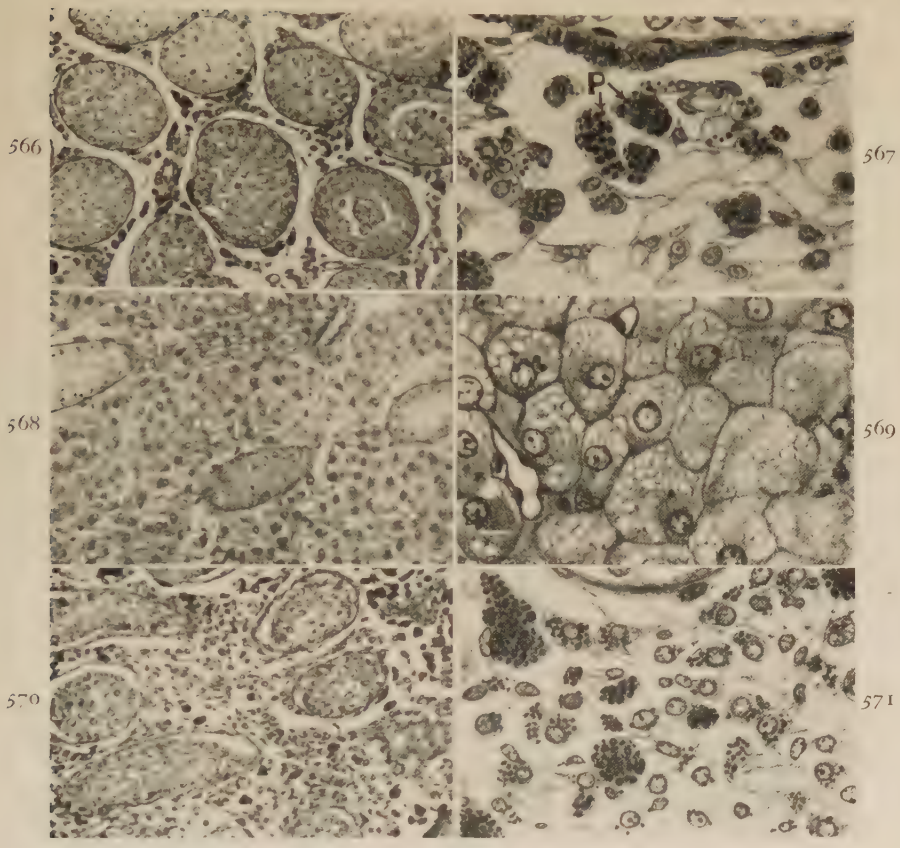
VI. SEASONAL VARIATIONS

Of special interest is the alternate hypertrophy (with or without hyperplasia) and atrophy of the interstitial cells which occur regularly in many adult animals that exhibit a marked annual sexual cycle. This subject has been approached largely from the point of view of the relationship between these variations in the activity of the interstitial cells and the development of secondary sex characters, rutting behavior and spermatogenesis. The literature is well summarized by Pellegrini (1925). In addition to the references there given, the extensive report on frogs and toads by Champy (1913), the recent studies by Aron (1924) also on frogs and toads and by van Oordt (1924) on fishes may be mentioned.

The extent to which these changes occur with the annual cycle is well shown in the woodchuck (*Marmota monax*), as represented in Figures 566 to 577. Not only is there the relative difference shown in the low power views (Figs. 566, 567 and 570), which are all photographed on the same scale, but also as great absolute changes occur because the entire testis enlarges as much as eight times during interstitial cell hypertrophy. When atrophy sets in (Fig. 570) the whole testis is reduced again to its original size, although the tubules are filling up with germinal cells in early stages of spermatogenesis. The more detailed views before (Fig. 567), during (Fig. 569) and after hypertrophy (Fig. 571) give a general idea of the radical changes that take place in the interstitial cells. Cytological details are shown in Figures 572 to 577, which are all drawn to the same scale, except Figure 575 (see explanation accompanying plate). The pigment cells (marked "p" in these cell groups) have already been referred to (p. 1220). A new crop of cells, like Figure 577p, is formed each year as a result of midsummer atrophy of the interstitial cells. The enormous amount of pigment formed causes the entire testis to assume a dark brown color. For further details see Rasmussen (1917, 1918).

As already stated in the section on the origin of interstitial cells, it appears that they may arise from spindle-shaped cells not unlike fibroblasts and return again to that stage.

Pellegrini (1925), from a review of the literature and a histological study of the bat, hedgehog, mole, sparrow, drake, lizard and tortoise (in all of



FIGS. 566–571.—Photographs of interstitial cells of the testis of adult woodchucks. Carnoy's fixative 4μ. Iron hematoxylin. 567, 569 and 571 retouched from light prints. Pigment shown as black granules. ×500. 566, 568, and 570 ×125.

FIG. 566.—During late autumn and early winter. Low power view. Tubules filled with cells in early stages of spermatogenesis. Some shrinkage of interstitial tissue from tubules. Black cells are the highly pigmented cells marked P in Figure 567.

FIG. 567.—Higher power view of interstitial tissue from same slide as Figure 566. Large group of highly pigmented cells in region marked P. Some of them are closely applied to the tubule.

FIG. 568.—During spring (April and May) at the close of the rutting season. Tubules empty and smaller. Interstitial cells maximal and crowding the enlarged intertubular space.

FIG. 569.—Higher power view of interstitial cells from same slide as Figure 568. Relation to capillaries well shown. A few pigment granules in some cells. Vacuoles represent lipoids dissolved out.

FIG. 570.—During late July and early August, after atrophy of interstitial cells. A new crop of large highly pigmented cells is evident. Tubules filling up with cells in early stages of spermatogenesis.

FIG. 571.—Higher power view of atrophic interstitial cells from same slide as Figure 570 showing more or less pigment in nearly all cells.

FIGS. 572–577.—Camera lucida drawings of individual representative interstitial cells and cell groups from adult woodchucks showing in greater detail and more diagrammatically the changes involved in the cycle shown in Figures 566–571. All figures (except 575) fixed in natural formalin and potassium dichromate. 3μ. Acid fuchsin and methyl green stain. Lipoid shown as vacuoles, pigment as large gray spherules, secretion-like fuchsinophil granules as the larger black spots and mitochondria as small round black granules. × 1000.

FIG. 572.—A, B, C, ordinary interstitial cells during winter (hibernation). P, highly pigmented cells showing some of the pigment granules fusing into larger globules and nucleus crowded to periphery of cell.

FIG. 573.—A, enlarging cell during early spring awakening. P, typical highly pigmented cells of this stage.

FIG. 574.—Late spring at close of breeding season. A, hypertrophied cell containing in addition to the usual constituents enlarged fuchsinophil granules in the central region. P, atrophic pigment cell. Most of these cells have disappeared by this time. Very large globules of pigment are characteristic.

which the testis presents marked physiological periodicity) and of the cat, dog, rabbit, guinea pig, pigeon and rooster (in which the testis is more or less continuously active), concludes that the spermatogenic function is independent of demonstrable changes in the interstitial cells, although in general the apparent stage of increased activity of the latter is simultaneous with the presence of mature spermatozoa.

During the period of sexual repose the interstitial cells are apt to be atrophic, while during sexual activity they tend to hypertrophy and may also increase in number.

On the whole, however, there is too much variability to permit any generalizations on the relation of the interstitial cells to the secondary sex manifestations, although in many cases there exists a close parallelism. Histological studies can never settle these questions independent of physiological and biochemical data. The parallelism may be entirely secondary and not necessarily evidence of causal relationship.

VII. HIBERNATION

Hibernation, while a normal physiological process, is accompanied by a radical reduction in the metabolic processes, especially in homoiothermal animals and by more or less inanition (p. 1237). These factors, in addition to the sexual quiescence, might naturally be expected to influence the morphology of the interstitial cells and other tissues of the body. Von Hansemann (1895), in fact, reported that during hibernation of the European marmot there was no spermatogenesis and that the interstitial cells were reduced to nothing but a few spindle-shaped cells, in comparison with the abundant and large epithelial-like cells of May and June, when the animal is active. Ganfini (1903), however, concluded that there was only a reduction in the size of the cells and not a decrease in number. Various conflicting opinions and observations have since been brought forth mostly in connection with the frog, toad, hedgehog and marmot. The literature is reviewed in detail by Rasmussen (1917) and more recently by Pellegrini (1925).

FIG. 575.—Same stage as in Figure 574 to show the arrangement of cells in "nests" as occurs in about half of these animals at this period. Relation of cells to blood vessels shown. Cell groups purposely accentuated by selecting a region where shrinkage by the fixer (Carney's) has occurred. Iron hematoxylin. × 250.

FIG. 576.—Cells in early stages of atrophy during mid-summer (July).

FIG. 577.—Final atrophic types in early autumn. Only a few remain as large as A. The processes on B and C become less evident with time (Figure 572). Mitochondria are about the only specific granules left except for pigment and this disappears from the smallest cells. P, a typical large highly pigmented cell of this period. Mitochondria are shown between the pigment granules.

The data as a whole show no specific effect of hibernation on the interstitial cells of the testis. They generally remain about as they were before dormancy set in. Von Hansemann's conclusions, which have been widely quoted, may evidently be attributed to his failure to examine the testis just before hibernation commences. There may be a gradual hypertrophy during winter sleep, especially in toads and frogs (Friedmann, 1898; Champy, 1913; Aron, 1924). In the woodchuck there is only a very slight change. A little enlargement of the cells takes place during winter sleep. Many cells remain apparently unchanged in size, although they may round-out more. Those that do enlarge are richer in mitochondria and lipoids. It is after waking up in the spring that marked changes take place, as already discussed (p. 1215). Certainly there is no sudden change at the onset of hibernation, as was emphasized by Mann (1916) who also could see no definite correlation between the hibernating state and the sex glands in the thirteen-lined ground squirrel (*Citellus tridecemlineatus*).

VIII. CAPTIVITY

It is a well-known fact that many animals kept in captivity do not breed. Branca (1904*a*) finds that in the axolotl there is marked atrophy of the testis with an enormous development of the interstitial cells; but in captive lemurs, which were also sterile, he found no change in the volume of the testis as a whole, nor in the tubules, and no interstitial cell hypertrophy.

IX. IN CERTAIN ABNORMALITIES

1. *Cryptorchidism:*

The general opinion among observers has been that the interstitial cells of abdominally retained testes are usually much more prominent than in normal scrotal organs (see, for example, Hanes, 1911, and Goddard, 1920). Evidently the shift in relative values occasioned by the undeveloped or atrophic condition of the tubules has led investigators astray because the recent studies by Moore and Oslund (1924), and especially the quantitative work of Bascom (1925), have failed to show an absolute increase in the case of sheep and swine; and yet it has been the pig that has most often been the subject of researches along this line. The interstitial cells remain perfectly normal morphologically in the opinion of most investigators. Uffreduzzi (1910), however, claims that in cryptorchid testes they do not contain exactly the same kinds of granules by osmic acid and Ciaccio's technique.

Benda (1921) says that in inguinally retained testes there are usually very few interstitial cells. In cattle, however, Bissonnette (1926) finds that testes retained at the upper end of the scrotum contain relatively a supra-abundance of interstitial cells essentially as in abdominal testes.

The functional significance of these ectopic testes, in which the germinal epithelium has invariably greatly degenerated without producing any noticeable change in the secondary sex characters, has been much discussed. The most recent paper on the surgical aspect of the subject by Wangensteen (1926) may be referred to for the literature. Oslund (1926*a*) has also just discussed the cryptorchid testis with reference to

hormone production. Frequently there are some spermatogonia left so that if the testis is replaced in the scrotum with its circulation undisturbed, spermatogenesis may be re-established. The entire absence of spermatogenic cells may finally result after long retention in the abdomen, but Sertoli cells are very resistant so that those who oppose the idea that the persistence of male characteristics is due to the interstitial cells can usually fall back upon the Sertoli cells as possible producers of the testicular secretion. However, Thorek (1924, pp. 70 to 76) seems to have demonstrated a human case with marked libido and secondary sex characters but with absolutely no epithelium left in the tubules and with abundant interstitial cells. Whitehead (1908) also thought that in a cryptorchid horse with unusually well-developed male habitus the Sertoli cells were also so degenerated as to be ruled out of consideration. Bouin and Ancel (1904*d*) claim that in cryptorchid pigs they found male-appearing animals without even Sertoli cells in the tubules but with apparently an over-abundance of interstitial cells.

2. *Eunuchoidism:*

This abnormality includes a variety of conditions where the individual shows to a milder degree a variable number of the symptoms following total castration. The testicular changes are exceedingly variable. More or less deficiency in the interstitial cells, either from arrested development or atrophy, has been noted (Thorek, 1924). But there is a sad lack of reliable data, especially of a quantitative nature, from which to judge how much correlation exists between various eunuchoid conditions and the morphology of the interstitial cells.

3. *Hermaphroditism:*

In mammals pseudo-hermaphroditism masculinus externus, i.e., where the external features are predominantly male, the testes are usually retained and have the structure of cryptorchid organs. Keufsler (1920) considers that the apparent increase in the interstitial cells is only relative, for the reason that the tubules have greatly atrophied. Gould (1923) describes the interstitial cells of the testis of a female-appearing pig with lateral hermaphroditism as being almost twice the normal diameter and relatively more abundant than normally. For a classical discussion of the literature, see Lipschütz (1924) on the subject of "intersexuality."

Pick's (1914) monograph on true hermaphroditism gives histological data on the ovo-testis in pigs and a few cases in man. The interstitial tissue in most cases are supra-abundant as compared with the amount of tubular tissue.

4. *In various diseases:*

The scattered observations on the condition of the interstitial cells in various diseases are difficult to summarize. It seems well established that there is an increase (possibly in most cases only apparent, due to tubular degeneration as Koch (1910), in particular, emphasized years ago and as shown by Slotopolsky and Schinz (1925*a*) recently by means of quantitative methods) in the number of the interstitial cells in chronic cachectic conditions, such as chronic phthisis, cancerous and syphilitic cachexia and pernicious anemia (von Hansemann, 1985; Cordes, 1898; Thaler, 1904; Kasai, 1908; Kyrle, 1911, 1920; Mita, 1914; Lespinasse, 1922; Berberich and Jaffé, 1922; and others). The reports quite generally show that not all individuals are thus affected. Cordes noted it in only 7 out of 12 cases of tuberculosis. Kasai observed it also in tuberculosis in only 17 out of 22. In chronic circulatory diseases, on the other hand, any noticeable change is rare (see Lubarsch p. 1232).

Ancel and Bouin (1905) and Bouin and Ancel (1905*a*) claim to have found interstitial cell hypertrophy in acute tuberculosis and pneumonia and in experimental infections, although not as regularly nor to the extent seen in chronic diseases, but Kasai

denies this. Mathieu (1808) says they increase as a consequence of fever, while Mills (1919) generally found no change in influenza. In a few cases there was some increase. Morgenstern (1923) finds especially marked reaction in typhus. It is evident as early as the seventh and eighth day and disappears after four or five weeks. The interstitial cells increase in number, lose lipoid and take on phagocytic properties. Additional literature may be obtained from Morgenstern's report. Berberich and Jaffé (1922) claim that the reaction to acute diseases in young men is different from what it is in old men. In the former the interstitial cells generally increase while in the latter they tend to decrease.

Mott (1919) states that they increase in those cases of dementia praecox in which the germinal epithelium is degenerating. In fact, the general principle seems to hold that interstitial cell hypertrophy or hyperplasia generally, though not invariably, accompanies secondary degeneration of the seminal epithelium (Dürch, 1907; Benda, 1921; Oslund, 1924*c*; and others).

Battaglia (1925) calls attention to the possibility in pathological cases of lipoid-laden cells of connective tissue origin being mistaken for true cells of Leydig and that these types of cells, while similar in many respects, are not identical. Lubarsch (1921) claims that these hypertrophied connective tissue cells contain some hemosiderin and that they belong with the macrophages. He mentions their presence in the testis in endocarditis and marasmus (see also Koch, 1910). Hedinger (1920) reports a case of chorionepithelioma of the right testis which was removed. At death, one month later, the left testis had a great increase in the interstitial cells, although the tubules were normal, showing that the interstitial cells may increase under certain conditions in the absence of tubular degeneration.

Tumors composed of interstitial cells have been reported by several men, especially by Chevassu (1906), Kaufmann (1911) and Kunze (1922*b*). The literature is reviewed by Kunze who found that interstitial cell tumors are common in old dogs where they constitute half of all tumors found in the testis. The cells of these tumors frequently contain glycogen but lack crystalloids.

In recent years attention has been directed more definitely towards chemical changes in the interstitial cells. Thus Ishibashi (1919) reports more than the normal amount of cholesterol esters in some diseases. Jaffé (1921) found definite hyperplasia of the interstitial cells and an increase in the lipoid content in constitutional inferiority and in marasmus. Oppermann and Jaffé (1924) found a loss of lipoid in children dead from chronic diseases, while Lotz and Jaffé (1924) decided there was an increase in phosphatides and cerebrosides in diabetes mellitus. An extensive tabulation of the reaction of the crystalloids in the testis in various diseases is given by Bukhofzer (1924).

Brack (1922) calls particular attention to the lack of correlation between the amount of interstitial cells in pathological cases and the degree of development of secondary sex characters. He claims there may be a total lack of interstitial cells with no obvious effect on sex characters and an over-abundance with no undue prominence of masculinity. For detailed volumetric data on the percentage of interstitial cells, connective tissue and germinal epithelium, etc., in the testes of a number of cases of psychopathia sexualis, see Slotopolsky and Schinz (1925*a*).

X. EXPERIMENTAL MODIFICATIONS

1. *Vasectomy:*

Although some of the earlier experiments on vasectomy, such, for example, as those by Shattock and Seligmann (1904), left the testis essentially normal, such investigators as Bouin and Ancel (1903, 1904, see also

Ancel and Bouin, 1904), Tandler and Gross (1913), Steinach (1920), Sand (1921), Thorek (1924), Berblinger (1921), Kuntz (1920, 1921), Myers (1915), Tiedje (1921), Massaglia (1920), and others have generally found an initial atrophy of the germinal epithelium but survival and in most cases hypertrophy or hyperplasia of the interstitial cells after ligation or resection of the ductus deferens in adults. The animals remain normal or show increased vigor for some time. Senile animals become temporarily younger in appearance and activity. Macht and Teagarden (1923) found that ligated rats learned to walk the tight rope in one-third the time required by normal rats. Ligated rats were also stronger than normals for six to eight weeks, after which they weakened to normal. The germinal epithelium frequently regenerates again.

Myers (1915) concluded that this interstitial cell increase is only apparent. The quantitative data of Romeis (1921), Stieve (1925), Slotopolsky and Schinz (1925) bring this out more clearly even when there is an absolute loss. From the work of Mottram and Cramer (1923), Moore (1923), Moore and Oslund (1924), Moore and Quick (1924), Oslund (1924), Warwick (1925), Humphrey (1926) and Wangensteen (1927) it is evident that vasectomy of itself has no detectable morphological effects on the interstitial cells. Some other factors must have entered into some of the experiments. Nonidez (1924*b*) says that only occasionally is there an absolute increase in the interstitial cells.

Ligation of the vasa efferentia in rats has also been found to cause degeneration of the tubules but no interstitial cell hypertrophy (Oslund, 1926*b*). In the human Simmonds (1921) reported that in only one out of 39 cases of long-standing stricture of the vas deferens was there interstitial cell increase.

In young animals this operation may result in infantile testes both as to germinal epithelium and interstitial cells. Such animals look like castrates (Bouin and Ancel, 1904*c*).

For a sane view of the application of this procedure to cases of impotency, etc., in man, see Sand (1922). Further details may be obtained from Lipschütz (1924) and Oslund (1924).

2. *Partial castration:*

The occurrence of hypertrophy of the interstitial cells of the remaining testis after removal of one, or of one testis and part of the other, has been frequently observed. This subject is thoroughly discussed under the title of "compensatory hypertrophy" by Lipschütz (1924) under whose direction much work of this character has been done. Pezard (1918) in the fowl and Lipschütz (1924, 1925) and coworkers in mammals, find that a very small fragment (2 per cent in guinea pigs and about 6 per cent in the chicken) of one testis is enough to prevent castration symptoms if the

interstitial cells are normal. The latter author mentions a rabbit in which the Sertoli cells were numerous and yet castration effects had not been prevented, but the interstitial cells were infantile. In evaluating all this type of evidence it must always be kept in mind that it is possible for dysfunction to occur without any morphological changes being observable in the cells involved.

The hypertrophy frequently takes place in the neighborhood of degenerated tubules. The upper pole of the mammalian testis seems to have a greater tendency to interstitial cell hypertrophy than the lower pole. Section of both testes near the lower pole but without removing any portion of either organ also results in hypertrophy. This phenomenon, and the fact that hypertrophy takes place in the presence of more than the necessary amount of intact testicular tissue, are used as arguments by Lipschütz against the idea that the hypertrophy is compensatory for endocrine purposes. Moore (1924) also argues against compensatory hypertrophy of the interstitial cells in a true sense.

Since a minimal amount of interstitial or other testicular tissue suffices to maintain masculinity, Pezard (1920, 1922) and Lipschütz (1924) have attempted to apply the "all-or-none" law to the endocrine function of the testis. Much evidence against this view has been presented by Schafer (1926). Experiments on partial castration are interpreted by Aron (1925) to indicate that such a law does not hold for the frog.

3. *Testicular grafts:*

The summaries by Lespinasse (1922), Thorek (1924), Lipschütz (1924) and other sources indicate that in successful "takes" in man and higher animals, the interstitial cells are generally prominent and normal in appearance, whereas the germinal epithelium tends to degenerate. The Sertoli cells usually survive. In the latest report by Moore (1926*b*) on 193 grafts of whole rat testes implanted in different situations in both normal and castrated males and females, 114 grafts were recovered after one to six and one-half months. The interstitial cell reaction in this series was inconstant. Some showed hypertrophy and others did not. Only grafts in the scrotum produced spermatozoa.

Since the application of this method and of vasoligation to human surgery has had much unpleasant notoriety, it is probably not out of place to refer to the opinion of Hoskins (1925), who is a most competent critic of endocrinology. After reviewing the literature on testicle grafts, he concludes: "The foregoing observations leave no room for reasonable doubt that testicle grafting is capable of producing definite somatic changes and definite erotization. They seem to lay upon the conservative surgeon the duty of accurate determination of the best methods and of the possibilities and limitations of the procedure."

4. Displaced testis:

The ectopic testis, even with nerve and blood supply intact, is atrophic, especially in animals with a closed scrotum. The germinal epithelium is degenerated, but the interstitial cells are normal or hypertrophic. Young testes are arrested in development. Replacement in the scrotum, if degeneration has not gone too far, results in return to normal (Moore, 1926*c*). A paper by Wangensteen (1927) may be consulted for a comprehensive review of the entire question.

Moore (1926*a*) finds that if the testis is placed subcutaneously it may remain practically normal. This observation adds one more link to the chain of evidence which Moore and co-workers, as well as Fukui (1923), have secured to show that it is undoubtedly the higher temperature of the surroundings that causes these changes in the displaced testis. This was also suggested by Crew (1922) with reference to the abnormality of undescended testes.

5. Temperature of environment:

Steinach and Kammerer (1920) caused an increase in the interstitial cells by keeping animals under high temperature for a long time. Fukui (1923) in the rabbit and Moore (1924*b*) in the guinea pig showed that direct application of heat (47 to 48°C. for ten to twenty minutes) to the scrotum causes degenerative changes in the tubules but with little or no effect on the interstitial cells. On the other hand, Stieve (1923*b*) found that keeping mice for several weeks at 37°C. produced considerable atrophy of the interstitial cells.

6. Roentgen ray:

It is evident that the interstitial cells are much more resistant to roentgen rays than any of the intratubular elements except Sertoli cells. This has been abundantly shown by: Bergonié and Tribondeau (1904, 1905), Villemin (1906), Regaud and Dubreuil (1907), Herxheimer and Hoffmann (1908), Simmonds (1909), Kyrle (1911), Barratt and Arnold (1911), Tandler and Gross (1913), Hüssy and Wallart (1915), Nemenov (1916), Hooker (1922), Ancel and Bouin (1923), Benoit (1924), Schinz and Slotopolsky (1925), and some others. Doses sufficient to produce marked tubular degeneration have either no effect on the interstitial cells, or cause them to increase in number. The increases noted are occasionally enormous. Yamakawa (1925) finds that in the cat very strong doses damage the interstitial cells before the Sertoli cells are affected. Light (substerility) exposures, according to Parkes (1925), may stimulate the germinal epithelium of male mice so as to change the sex ratio of the off-spring when mated to normal females, no interstitial cell changes being observed. Regaud and Dubreuil (1908) report that the tests of young

rabbits are relatively immune to roentgen rays. This does not seem to be the case in dogs, however (Schinz and Slotopolsky, 1924).

Thorek (1924) found that proper doses were beneficial to testicular grafts in monkeys, possibly by preventing excessive leucocytic invasion and by stimulating vascularization of the graft.

If the effect on the germinal epithelium has not been too great, regeneration takes place and the interstitial cells return to normal. The size of the dose necessary to produce a given degree of testicular reaction was found by Hooker (1925) to be so variable, in the rat at least, that even after numerous experiments he was unable even to approximate the dose that would give a particular degree of reaction in a given animal. Hooker further states that small doses to immature animals result in testes composed essentially of Sertoli cells and interstitial cells, yet such animals develop normally, to all appearances.

This line of investigation, like many others, suffers from a lack of application of quantitative methods in estimating the degree of reaction, especially by the interstitial cells.

7. *Radium:*

Mottram and Cramer (1923) report that radiating the testis of young rats resulted in the animals becoming excessively fat with testes from one-half to one-fifth the normal size. In general, the tubules showed complete cessation of spermatogenesis, although limited areas may remain normal. The interstitial cells were greatly increased. The hypophysis also showed changes which were secondary to the testicular abnormality.

8. *Reaction to healing of testicular wounds:*

Maximow (1899) studied very carefully the histological changes that take place at various intervals after producing aseptic wounds with sharp knives and scissors and hot needles in the testes of dogs, cats, rabbits, mice, rats, guinea pigs, frogs and tritons. After a couple of days the interstitial cells at the margin of the necrotic focus show active caryokinesis. Then follows hypertrophy so that the tubules become separated by a wide expanse of granular tissue. Both the nucleus and cytoplasm increase in size. The nucleus becomes hyperchromatic. Lipoid globules tend to disappear from the cytoplasm of some cells and to become arranged into irregular clumps, although in some cells no such changes occur. In the dog and cat many interstitial cells atrophy and are removed by phagocytic leucocytes that rapidly invade the area.

9. *Over-feeding:*

Stieve (1921*b*, 1922, 1923*a*, 1926) claims that the interstitial cells are greatly increased in number and in size in the fattened domestic gander.

Since such males become sterile for a time and lose secondary sex characters, he argues that the secondary sex characters depend upon the presence of normal germinal epithelium and not upon the interstitial cells. In the common house mouse he also finds that the interstitial cells attain a maximum in the fattest animals. These observations have, however, not received confirmation. The most recent and at the same time the most reliable data are those supplied by Saller (1926) who used more accurate quantitative methods in evaluating the absolute and relative amounts of interstitial and germinal tissues in white mice. Fattening produced variable results. While the interstitial cells as well as the germinal tissue may be damaged and reduced in some individuals, and in others show practically no change or only a little enlargement, occasionally the proportion of interstitial tissue to germinal tissue may become 1:6 as against 1:10 in the normal. The literature is critically reviewed by Saller. The storage of excessive fat, as it occurs in hibernating mammals like the marmot, certainly does not influence the interstitial cells.

Chuin and Machida (1922) find that feeding lanolin, egg yolk, lard, butter, cod liver oil, olive oil and some vegetable oils produces changes in the testis as seen in senility, such as degeneration of the epithelium, thickening of the basal membrane, with an increase in interstitial cells during the first six months, after which no interstitial cell changes are seen; but it is not clear from the abstract whether these fats were fed in addition to regular food or alone.

10. *Inanition (total or with water only):*

Jackson (1925) summarizes the published observations on this point by saying that the interstitial cells appear variable. In some cases they are atrophic, in others hypertrophied, especially upon re-feeding after inanition. The fatty content appears to belong to the "permanent" or "sessile" lipoids which are relatively unaffected by starvation. Thus Kunze (1922*a*), who investigated a number of mammals, including man, concluded that the amount of fatty inclusions was independent of the general nutrition of the body.

To what extent the interstitial cell increase seen in chronic cachexia in man, as discussed on page 1231, is due to undernutrition is difficult to say. In all probability in some cases this may be a factor. The general results of a number of investigations bearing directly on the subject may be briefly mentioned.

Cordes (1898) noted an increase in the interstitial tissue in chronic malnutrition. As already mentioned, Jaffé (1921) reported interstitial cell hypertrophy and an increase in lipoids in marasmus. In atrophic infants Mattei (1914) sometimes found pigmentation, at other times slight atrophy. In famine-stricken children Stefko (1923, 1924) and Maslowsky (1923) found an increase in the interstitial cells.

Simonowitsch (1899) found edema in the interstitial tissue in rabbits and guinea pigs, especially in chronic inanition, with distinct evidence of fatty infiltration in the interstitial cells, as early as five or six days after re-feeding. In the mouse and white rat Monterosso (1912) and Monterosso and Schlatter (1911) claim that the lipoid disappears immediately upon the onset of inanition. Stieve (1923*b*) gives figures which indicate that the interstitial tissue of starved mice decreases to about one-fourth of what it is in well-nourished animals, due to loss of lipoid.

On the other hand practically negative results were found by Beeli (1908) in the cat, Siperstein (1921) in the white rat, Yamasaki (1921) in white mice, Lindsay and Medes (1926) in the guinea pig, Traina (1904) and Okuneff (1922, 1923) in rabbits.

Upon re-feeding after inanition in growing rats definite hyperplasia and hypertrophy of the interstitial cells were noted by Siperstein (1921). Saller (1926) also applied quantitative methods to the testis in both acute and chronic inanition. In acute inanition the decrease in the interstitial cells was greater than in chronic inanition. The ratio of interstitial tissue to germinal tissue in acute cases may become as little as 1:26 and in the chronic 1:13 or 1:14, as compared with the normal ratio of 1:10.

11. *Deficiency diets:*

Vitamin B deficiency especially seems to cause testicular atrophy. Only a few investigators mention the condition of the interstitial cells. Thus Osborne and Mendel (1918) and Allen (1919) in the rat, Novaro (1920), Gotta (1923) and Simnitzky (1926) in the pigeon and McCarrison (1921) in pigeon and monkey (more pronounced in the pigeon) note hyperplasia or hypertrophy or both in the insterstitial cells. This is especially pronounced if there is also deficiency in vitamin A (McCarrison, 1921; Meyerstein, 1922), although deficiency in vitamin A alone has no specific effect on the testis (Parkes and Drummond, 1926). Portier (1920) states, however, that in the pigeon the intertubular connective tissue increases to such an extent that the interstitial cells atrophy. Finlay (1921), in beriberi of pigeons and fowls, found some swelling of the nucleus and loss of lipoid, while Houlbert (1919) mentions increased pigmentation. Parkes and Drummond (1925), in a recent study, indicate that the interstitial cells hypertrophied in only one rat out of half a dozen.

Lack of *vitamin* C, according to Lindsay and Medes (1926), produces in the testis of guinea pigs degenerative changes in the tubules but the only change in the interstitial cells that could be noted was a slight decrease in the granular appearance of the cytoplasm.

Yamasaki (1923) could see very little change in the interstitial cells of white mice on a diet free from vitamins B and C, although the tubules were degenerated. In the absence of vitamins A, B and C, this investigator sometimes found an increase and at other times a decrease in the number of interstitial cells. The cells may be smaller or larger than normally. No change in the lipoid content was evident. Tsuji (1920) fed rats on food that was poor in vitamins without producing any change in the interstitial cells in spite of tubular degeneration.

Vitamin E is of special interest on account of its sex specificity. Mattill and Carman (1923) reported that a milk diet, and even a milk diet to which kidney and liver tissue has been added and which should contain adequate vitamin B and probably the other well-known vitamins, nevertheless caused tubular degeneration and an abundant proliferation of interstitial tissue. Evans (1925) and Mason (1925, 1926) have cleared up this problem considerably by finding that the lack of vitamin E in certain purified foods is responsible for the sterility in the male rat. Since they find degeneration of the germinal epithelium, the apparent increase in the interstitial cells is considered by Mason to be relative only. In advanced degeneration due to lack of vitamin E there is loss of sexual interest. Unilateral castration does not alter the rate of degeneration. For further discussion see Mattill and Clayton (1926). Further confirmation has also just been published by Kennedy (1926).

An exclusive meat diet in fowls was shown by Paul (1906), Houssay (1907) and Pezard (1919, 1920) to produce extensive testicular degeneration in many fowls. There are progressive sterility, loss of secondary sex characters and of the fighting instinct, indicating that all elements of the testis suffered. Some of the fowls remained normal. Paul also noted that an excessive meat diet in rats retards development of the male sex organs, but the histological appearance of the interstitial cells is not mentioned. Kennedy (1926) reports slight degeneration in the testis of rats on high protein diet, but says nothing about the interstitial cells.

Salt deficiency, according to Yamasaki (1923), produces no noticeable change in the testis of white mice. According to Kennedy (1926) a low calcium diet may produce slight testicular degeneration.

12. *Drugs and gland extracts:*

Of the drugs that affect the testes *alcohol* has received most attention. Bouin and Garnier (1900) and Ancel and Bouin (1905) made some observations which indicate that, in the final stages of alcoholic intoxication of several months' duration, atrophy of the interstitial cells may take place in rats. Stieve (1923*b*), in mice, also reports distinct atrophy with loss of lipoid and a fading-out of the cell boundaries. On the other hand, Kyrle and Schopper (1914) and Kostitch (1921) found interstitial cell hypertrophy accompanying alcoholic degeneration of the tubules. Kostitch adds that the hypertrophied interstitial cells commence to undergo pigmentary degeneration when tubular degeneration is more or less complete. Allen (1919), however, who examined the testes of rats made drunk every day for many weeks beginning at the age of twenty-eight days, could see no change in the interstitial cells, although the germinal epithelium was greatly degenerated. In human subjects Weichselbaum (1910) and Bertholet (1913) found hyper-

plasia of the interstitial cells in chronic alcoholic subjects with various degrees of tubular degeneration.

Massive doses of *iodine* administered subcutaneously to rabbits by Adler (1914) had about the same effect as alcohol; i.e., tubular degeneration and hyperplasia of the interstitial cells, the latter largely by means of amitotic cell division. Kostitch and Telebakovitch (1926) also report degeneration in the seminal tubules, but no changes in the interstitial cells, of white rats as a result of giving iodine by mouth.

Nicotine, according to Hofstätter (1923), administered to dogs, rabbits, cats, mice, rats, guinea pigs and chickens, generally causes tubular atrophy without interstitial cell increase. In immature animals and the young of mothers treated with nicotine, the testes are retarded in development.

Saponin given subcutaneously to cats is reported by Leupold (1921) to produce a loss of cholesterol in the interstitial cells of the testis and cortical cells of the suprarenals.

Thyroid extract, according to the work of Speidel (1925), greatly accelerates the rate of cell division in the mesenchyme from which the interstitial cells arise. Saller (1926) finds that feeding large doses of thyroid gland ultimately causes testicular atrophy, the interstitial tissue suffering most. Small doses also damage the germinal epithelium but increase the interstitial tissue relatively and probably also absolutely; however, the interstitial cells are not evaluated separately.

A particular influence of the *hypophysis* on the reproductive organs, especially of the male, has generally been accepted, largely because of the sexual disturbances accompanying disorders of this organ. The most specific experimental grounds for such a belief seem to consist of the effects of hypophyseal extracts as reported by Goetsch (1916). Goetsch found that feeding powdered anterior lobe of the hypophysis for eight to nine months to young rats hastened sexual development, accelerated growth of the testes and produced a moderate increase in the interstitial cells as compared with controls. Some influence of the hypophysis on the interstitial cells has also been inferred from the histology of the testis in acromegaly and gigantism as reported by Tandler and Gross (1913). Camus and Roussy (1922), on the contrary, found that the genital atrophy which has so regularly been associated with hypophyseal abnormality did not occur if the base of the brain was not disturbed during hypophysectomy. Smith (1926) likewise suggests that the sexual gland atrophy and adiposity in the rat are due primarily to the injury of the tuber cinereum and anterior lobe at the time of removal; and, further, that when the anterior lobe is harmed there is also rapid regression of the adrenals (involving chiefly the cortex) with accompanying structural changes in the thyroid. The injection of anterior lobe extract from bovine hypophyses into pituitariless rats brings about nearly normal conditions.

A great many workers have also called attention to the apparent close relationship between the cells of the *suprarenal cortex* and the interstitial cells, largely because of changes in their lipoid content and especially their cholesterol esters (Aschoff, 1924) under pathological and experimental conditions.

To go further would lead too far afield, since certain measures produce such widespread effects that many tissues are involved (including the interstitial cells of the testis), making it impossible to decide whether the interstitial cells are influenced directly or secondarily due to disturbance in some other organ. A case in point is the description of certain nuclear changes in the interstitial cells of the testis brought about by extirpation of the pancreas by Kraus (1921). Here the hypophysis, suprarenal, thyroid and pineal body, as well as other organs, were also affected.

13. *Abolition of the nerve supply to the testis:*

Kuntz (1919*a*, *b*) removed the inferior mesenteric ganglion and the nerves descending along the right spermatic artery of dogs. Both testes showed distinct degeneration of the germinal epithelium after three weeks and advanced degeneration after four weeks. The interstitial cells increased in size and in many places there was evidence of hyperplasia. Takahashi (1922) also found atrophy of the testis after excision of the lumbosacral part of the sympathetic trunk from the third lumbar to the first or second sacral ganglia in guinea pigs. The interstitial cells may hypertrophy, remain unchanged or atrophy.

Ceni (1909) claims that removal of the cerebral hemispheres of male ducks increases the interstitial tissue, but it is not clear whether the interstitial cells are involved or merely the connective tissue.

14. *Interference with the blood supply to the testis:*

Various experiments from the earliest ones of Griffiths (1895) on dogs to the most recent ones by Wangensteen (1927) show that ligation of the arteries or veins or both cause atrophy of the tubules and of the interstitial cells.

15. *Cross-breeding:*

Guyer (1912) crossed guinea and common fowls and Poll (1920) crossed pea and guinea fowls. In both cases the testes of the hybrids had degenerated tubules and an enormous increase in the interstitial cells.

XI. FUNCTIONAL SIGNIFICANCE

The morphology and the reaction of the interstitial cells under various physiological, pathological and experimental conditions, as summarized in the preceding pages, have been interpreted by many as indicating that in the higher animals these cells are intimately connected with the development

of the secondary sex characters and sexual behavior. Sand (1923) aptly remarks: "In reality no new points of vital importance have arisen which, with regard to mammals, can change the opinion that the Leydig cells have, besides their nutritive function, great, perhaps the very greatest, importance for the hormone production of the testis. Of this most of the modern scientists are certainly now convinced." A further discussion of this point will be found in this author's most recent publication (Sand, 1926, p. 272). A discussion of the place which such hormones hold in the general phenomenon of the determination of sex in animals is given by Swingle (1926). Lipschütz (1924, Chap. XI) also advances important points of view, a discussion of which is beyond the scope of this section. There are certainly many authentic cases of male characteristics persisting in the absence of all the germinal epithelium except Sertoli cells. The critical cases are those few in which the Sertoli cells are also lacking, such as those reported by Bouin and Ancel (1904*d*) in the pig and by Thorek (1924) in man (p. 1231) with a persistence of masculinity. If these observations are correct, one must assume that castration effects are due to the loss of something else besides the epithelium of the tubules. Since the ordinary connective tissue of the testis is not likely to be the seat of hormone production, the interstitial cells seem to be the most likely source.

The correlation of the presence, and time of appearance of the interstitial with the sterility and morphology of the free-martin in cattle, which has been carried out by Lillie (1917, 1923) and his students (Chapin, 1917; Willier, 1921; Bascom, 1923), points in the same direction.

Harms (1922), however, believes that the significance of these cells has been greatly exaggerated by Bouin, Ancel, Steinach, and others. His idea is that a pro-secretion is produced by the epithelium of the tubules and merely stored in the interstitial cells where it is transformed to the active secretion (incretion). Kitahara (1923) and Cejka (1923) also say something about the possibility of their altering chemical substances elaborated by the germinal epithelium. Berblinger (1921) holds the view that they merely store hormones produced by the germinal epithelium. Moore (1926*d*) in a recent careful review of the literature and his own extensive experience is inclined to the view that in the last twelve years there has been a decline in the emphasis placed upon the significance of the interstitial cells. Brack (1922) on pathological grounds denies that they have anything to do with determination or maintenance of the secondary sex characters. Stieve (1921 to 1923), Champy (1922), Fukui (1923*b*), Walker (1924) and some others definitely attribute the control of these processes to the germinal epithelium.

The mechanism is evidently complex and may involve other endocrine organs. Blair Bell (1916) goes so far as to say that the gonads play only a subservient part in these processes. While this may be true of lower vertebrates and animals still lower in the scale, certainly this is an unwarranted

statement for higher vertebrates. A recent review of the essential work on the inter-relations with the other endocrine organs will be found in Schafer's (1926) recent book. The general question is well discussed by Lipschütz (1924, pp. 103 to 104).

Various other functions of the interstitial cells of the testis have been proposed. Plato (1896), Lenhossék (189–), Friedmann (1898), Kyrle (1911), Kohn (1920), Tiedje (1921), Stieve (1921 to 1926), Champy (1922, at least below mammals), Scheunig (1923), van Oordt (1924), Walker (1924), Simnitzky (1926) and others consider them merely as trophic. Regaud (1901) thought he saw substances passing from the interstitial cells to the Sertoli cells. Von Bardeleben (189–) argued that they are young Sertoli cells which pass through the wall of the tubules to replace worn-out cells and Goldmann (1909) illustrates such a passage of cells into the tubules. However, Hanes (1911) with the same technique could see nothing of this sort. Voinow (1905) advanced the hypothesis that they render non-toxic substances that might otherwise injure the germinal epithelium. Koch (1910) concluded that they are merely supporting structures—being hypertrophied connective tissue cells. A highly theoretical mechanism involving the nervous system and blood circulation has recently been formulated by Schweizer (1925).

While investigations of sex reversal, sex intergrades, antagonism between sex organs, etc., have yielded many suggestive facts, the significance of the interstitial cells must still be deducted from inconclusive data and great reserve is therefore necessary in making generalizations. Comparable experiments have not been made over a sufficiently wide range of animals and quantitative data are all too scarce. Many pass judgment upon a few sections, not knowing even the weight or size of the entire organ. An enormous amount of individual variability may exist, so that data from a few animals are unreliable as an index of the species.

XII. BIBLIOGRAPHY

Adler, L. 1914. Über Jodschädigungen der Hoden. *Arch. f. exper. Path. u. Pharmakol.*, **75**, 362.

Allen, B. M. 1904. The embryonic development of the ovary and testis of the mammals. *Am. J. Anat.*, **3**, 89.

Allen, E. 1919. Degeneration in the albino rat testis due to a diet deficient in the water soluble vitamines with a comparison of similar degeneration in rats differently treated and a consideration of the Sertoli tissue. *Anat. Rec.*, **16**, 93.

Ancel, P., and Bouin, P. 1903. Histogénèse de la glande interstitielle du testicule chez le porc. *Compt. rend. Soc. de biol.*, **55**, 1680.

——— 1904. L'apparition des caractères sexuels secondaires est sous la dépendance de la glande interstitielle. *Compt. rend. de l'Acad. Sc.*, Paris, **138**, 168.

——— 1905. La glande interstitielle du testicule et la défense de l'organisme. II. Hypertrophie ou atrophie partielle de la glande interstitielle dans certaines conditions expérimentales. *Compt. rend. Soc. de biol.*, **58**, 554.

Ancel P., and Bouin, P. 1923. Les cellules séminales ont-elles une action sur les caractères sexuels? *Ibid.*, **89**, 175.

Aron, M. 1921*a*. Sur l'existence et le rôle d'un tissu endocrinien dans le testicule des urodèles. *Compt. rend. de l'Acad. d. Sc.*, Paris, **173**, 57.

——— 1921*b*. Sur le conditionnement des caractères sexuels secondaires chez les batraciens urodèles. *Compt. rend. Soc. de biol.*, **85**, 482.

——— 1921*c*. Sur la glande interstitielle du testicule embryonnaire chez les mammifères. *Ibid.*, **85**, 107.

——— 1924. Cycle séminal, cycle interstitiel et caractères sexuels secondaires chez *Rana esculenta et Rana temporaria*. *Ibid.*, **90**, 797 and 800.

——— 1925. Conséquences de la castration partielle chez *Rana esculenta*. Notion de l'hyperactivité compensatrice de la glande interstitielle. *Ibid.*, **92**, 1398.

Aschoff, L. 1924. The normal and pathological morphology of the suprarenals. In *Lectures on Pathology*. New York: Paul B. Hoeber, Inc., p. 101.

von Bardeleben, K. 1897. Beiträge zur Histologie des Hodens und zur Spermatogenese beim Menschen. *Arch. f. Anat. u. Physiol.*, Anat. Abt. Supplement Bd., 193.

Barratt, J. O. W., and Arnold, G. 1911. Cell changes in the testis due to X-rays. *Arch. f. Zellforsch.*, **7**, 242.

Bascom, K. F. 1923. The interstitial cells of the gonads of cattle with especial reference to their embryonic development and significance. *Am. J. Anat.*, **31**, 223.

——— 1925. Quantitative studies of the testis. I. Some observations on the cryptorchid testes of sheep and swine. *Anat. Rec.*, **30**, 225.

Bascom, K. F., and Osterud, H. L. 1925. Quantitative studies of the testicle. II. Pattern and total tubule length in the testicle of certain common mammals. *Anat. Rec.*, **31**, 159.

Battaglia, F. 1925*a*. Die Leydigschen Zellen und Ciaccios Lipoidinterstitialzellen. *Arch. f. path. Anat. u. Physiol. u. f. klin. Med.*, **257**, 662.

——— 1925*b*. Le cellule interstiziali del testicolo in condizioni spermimentali. *Haematologica*, **6**, 263.

Beeli, S. S. 1908. (Cited by Jackson, 1925.)

Beissner, H. 1898. Die Zwischensubstanz des Hodens und ihre Bedeutung. *Arch. f. mikr. Anat.*, **51**, 794.

Bell, W. B. 1916. *The sex complex*. New York: Wm. Wood and Co.

Benda, C. 1921. Bemerkungen zur normalen und pathologischen Histologie der Zwischenzellen des Menschen und der Säugetiere. *Arch. f. Frauenk. u. Eugenetik.*, **7**, 30.

Benoit, J. 1922*a*. Sur les cellules interstitielles du testicule du coq domestique. Evolution et structure. *Compt. rend. Soc. de biol.*, **87**, 1382.

——— 1922*b*. Sur une méthode permettant de mesurer la masse absolue du tissu interstitiel testiculaire. *Ibid.*, **87**, 1385.

——— 1922*c*. Sur les rapports quantitatifs entre le tissu interstitiel testiculaire, le tissu séminal et la masse du corps chez les oiseaux et quelques mammifères. *Ibid.*, **87**, 1387.

——— 1923*a*. Sur les modifications cytologiques des cellules interstitielles du testicule chez les oiseaux à activité sexuelle périodique. *Ibid.*, **88**, 202.

——— 1923*b*. Sur les variations quantitatives des tissus interstitiels glandulaire et non glandulaire dans le testicule des oiseau à activité sexuelle périodique. *Ibid.*, **88**, 205.

——— 1923*c*. Sur l'origine des cellules interstitielles dans le testicule du coq domestique. *Compt. rend. l'Acad. d. Sc.*, Paris, **177**, 412.

——— 1924. Action des rayons X sur le testicule du coq. *Compt. rend. Soc. de biol.*, **90**, 802.

Berberich, J., and Jaffé, R. 1922. Die Hoden bei Allgemeinerkrankungen (mit besonderer Berucksichtigung des Verhaltens der Zwischenzellen). *Frankfurt. Ztschr. f. Patb.*, **27**, 395.

Berblinger, W. 1921. Über die Zwischenzellen des Hodens. *Verhandl. d. deutsch. patb. Ges.*, **18**, 186.

Berger, L. 1922*a*. Sur l'existence d'une glande ovarienne homologue de la glande interstitielle testiculaire. *Compt. rend. Acad. d. Sc.*, Paris, **175**, 498.

——— 1922*b*. Sur l'existence de glandes sympathicotropes dans l'ovaire et le testicule humains; leurs rapports avec la glande interstitielle du testicule. *Ibid.*, **175**, 907.

Bergonié, J., and Tribondeau, L. 1904 and 1905. Action des rayons X sur le testicule du rat blanc [and other titles]. *Compt. rend. Soc. de biol.*, **57**, 400, 592; *ibid.*, **58**, 154, 282, 678, 1029.

Bertholet. 1913*a*. Action de l'alcoolisme chronique sur les organes de l'homme et sur les glandes reproductrices. Lousanne (cited by Lipschütz, 1924).

——— 1913*b*. Petit atlas manuel des altérations anatomopathologiques des organes dans l'alcoolisme chronique. Paris (cited by Lipschütz, 1924).

Bissonnette, T. H. 1926. The "high flanker" testis in cattle with its bearings on the problem of the scrotum and on that of the freemartin testis. *Anat. Rec.*, **33**, 47.

Blacher, L. J. 1926. The dependence of secondary sex characters upon testicular hormones in *Lebistes reticulatus*. *Biol. Bull.*, **50**, 374.

Boll, F. 1871. Untersuchungen über den Bau und die Entwickelung der Gewebe. *Arcb. f. mikr. Anat.*, **7**, 324 (footnote).

Boring, A. M., and Morgan, T. H. 1918. Lutear cells and hen-feathering. *J. Gen. Physiol.*, **1**, 127.

Boring, A. M., and Pearl, R. 1917. Sex studies. IX. Interstitial cells in the reproductive organs of the chicken. *Anat. Rec.*, **13**, 253.

——— 1918. Sex studies. XI. Hermaphrodite birds. *J. Exper. Zool.*, **25**, 1.

Bouin, P., and Ancel, P. 1903. Recherches sur les cellules interstitielles du testicule des mammifères. *Arcb. d. zool. expér. et gén.*, **1**, ser. 4, 437.

——— 1904*a*. Recherches sur la signification physiologique de la glande interstitielle du testicule des mammifères. Rôle de glande interstitielle chez l'embryon, les sujects jeunes et âges; ses variations fonctionelles. *J. physiol. et patbol. gén.*, **6**, 1012.

——— 1904*b*. La glande interstitielle a seule dans le testicule une action générale sur l'organisme. Démonstration expérimentale. *Compt. rend. de l'Acad. d. Sc.*, Paris, **138**, 110.

——— 1904*c*. L'infantilisme et la glande interstitielle du testicule. *Ibid.*, **138**, 231.

——— 1904*d*. Tractus génital et testicule chez le porc cryptorchide. *Compt. rend. Soc. de biol.*, **56**, 281.

——— 1904*e*. La glande interstitielle chez le vieillard, les animaux agés et des infantiles expérimentaux. *Ibid.*, **56**, 282.

——— 1905*a*. La glande interstitielle du testicule et la défense de l'organisme. I. Hypertrophie ou atrophie partielle de la glande interstitielle ou cours de certaines maladies chez l'homme. *Ibid.*, **58**, 553.

——— 1905*b*. La glande interstitielle du testicule chez le cheval. *Arcb. de zool. expér. et gén.*, **3**, ser. 4, 391.

Bouin, P., and Garnier, C. 1900. Altérations du tube séminifère au cours de l'alcoolisme expérimental chez le rat blanc. *Compt. rend. Soc. de biol.*, **52**, 23.

Brack, E. 1922. Zur pathologischen Anatomie der Leydigzelle. *Arcb. f. patb. Anat. u. Physiol. u. f. klin. Med.*, **240**, 127.

Branca, A. 1904*a*. Le testicule chez l'axolotl en captivité. *Compt. rend. Soc. d. biol.*, **56**, 243.

——— 1904*b*. Recherches sur le testicule et les voies spermatiques des lémuriens en captivité. *J. de l'anat. et de la physiol.*, **40**, 35.

Branca, A., and Basseta, A. 1907. Sur le développement du testicule humain. *Arch. gén. de chir.*, **1**, 116.

Bukofzer, E. 1924. Über das Verhalten der Krystalle und Krystalloide im Hoden bei den verschiedenen Erkrankungen und Altersstufen. *Arch. f. path. Anat. u. Physiol. u. f. klin. Med.*, **248**, 427.

Camus, J., and Roussy, G. 1922. Les fonctions attribuées à l'hypophyse. *J. de physiol. et de path. gén.*, **20**, 509.

Čejka, B. 1923. Eine Studie über die Genese und Funktion des Interstitiums auf Grund der Untersuchungen an seneseenten Hoden. *Arch. f. mikr. Anat.*, **98**, 524.

Ceni, C. 1909. L'influenza del cervello sullo sviluppo e sulla funzione degli organi sessuali maschili. *Riv. sper. di Frenatria*, **35**, 3.

Champy, C. 1913. Recherches sur la spermatogénèse des batraciens et les elements accessoires du testicule. *Arch. de zool. expér. et gén.*, **52**, 13.

——— 1922. Étude expérimentale sur les différences sexuelles chez les tritons (Triton alpestris Laur). *Arch. de morph. gén. et expér.*, Fasc. 8, 1.

——— 1923*a*. Réponse à Aron. *Compt. rend. Soc. de biol.*, **88**, 1186.

——— 1923*b*. Observations sur les caractères sexuels chez les poissons. *Ibid.*, **88**, 414.

Chapin, C. L. 1917. A microscopic study of the reproductive system of foetal freemartins. *J. Exp. Zool.*, **23**, 453.

Chevassu, M. 1906. *Tumeurs du testicule.* Paris.

Chuin, T., and Nachida, S. 1922. Über Veränderungen des Hodens bei der Fütterung mit verschiedenen Fettsubstanzen. *Ni. Byor. Gak. K.* Tokyo, **12**, 105. Abstract in *Jap. J. Med. Sc.*, Abstracts, **2**, 169 (1925).

Ciaccio, C. 1910. Contributo alla distribuzione ed alla fisiopathologia cellulare dei lipoidi. *Arch. f. Zellforsch.*, **5**, 235.

Coley, W. B. 1908. The treatment of the undescended or maldescended testis associated with inguinal hernia. *Ann. of Surg.*, **48**, 321.

Cordes, H. 1898. Untersuchungen über den Einfluss acuter und chronischer Allgemeinerkrankungen auf die Testikel, speziell auf die Spermatogenese, sowie Beobachtungen über das Auftreten von Fett in den Hoden. *Arch. f. path. Anat. u. Physiol. u. f. klin. Med.*, **151**, 402.

Courrier, R. 1921*a*. Sur l'existence d'une gland interstitielle dans le testicule des poissons. *Compt. rend. Soc. de biol.*, **85**, 939.

——— 1921*b*. Sur le conditionnement des caractères sexuels secondaires chez les poissons. *Ibid.*, **85**, 486.

——— 1922. Étude préliminaire du déterminisme des caractères sexuels secondaires chez les poissons. *Arch. d'anat., d'hist. et d'embry.*, **2**, 115.

Crew, F. A. E. 1922. A suggestion as to the cause of the aspermatic condition of undescended testis. *Jour. Anat.*, **56**, Part 2, 98.

Diamantopoulos, S. 1921. Über die Hypoplasie der Hoden in der Entwicklungsperiode. *Ztschr. f. ang. Anat. u. Konstit.*, **8**, 117.

Dürch, H. 1907*a*. Über die Zwischenzellenhyperplasie der Hoden mit Atrophie des Organes unter Vorweisung von Präparaten. *München. med. Wchnschr.*, **54**, 1154.

——— 1907*b*. Über die Zwischenzellenhyperplasie des Hodens. *Verhandl. d. deutsch. path. Ges.*, **11**, 130.

Duesberg, J. 1918. On the interstitial cells of the testicle in Didelphys. *Biol. Bull.*, **35**, 175.

Evans, H. M. 1925. Invariable occurrence of male sterility with dietaries lacking fat soluble vitamine E. *Proc. Nat. Acad. Sc.*, **11**, 373.

Evans, H. M., and Schulemann, W. 1914. The action of vital stains belonging to the benzidine group. *Science*, **39** N.S., 443.

Felix, W. 1912. The development of the urinogenital organs. In Keibel and Maul, *Manual of Human Embryology*, **2**, 895.

Fell, H. B. 1923. Histological studies on the gonads of the fowl. I. The histological basis of sex-reversal. *Brit. J. Exp. Biol.*, **1**, 97.

Finlay, G. M. 1921. An experimental study of avian beriberi. *J. Path. and Bact.*, **24**, 175.

Firket, J. 1920. Recherches sur l'organogénèse des glandes sexuelles chez les oiseaux. *Arch. de Biol.*, **30**, 393.

Franckenberger, Z. 1922. Zur Frage der funktionellen Bedeutung der Hodenzwischenzellen. *Anat. Anz.*, **55**, 545.

Friedmann, F. 1898. Beiträge zur Kenntniss der Anatomie und Physiologie der mannlichen Geschlechtsorgane. *Arch. f. mikr. Anat.*, **52**, 856.

Fukui, N. 1923*a*. On the action of heat rays upon the testicles. An histological, hygienic and endocrinological study. *Acta Scol. Med. Univ. Imp. Kioto*, **6**, 225.

——— 1923*b*. Action of body temperature on the testicle. *Japan Medical World*, **3**, 160.

Ganfini, C. 1902. Struttura e sviluppo delle cellule interstiziali del testicolo. *Arch. Ital. di Anat. e di Embri.*, **1**, 233.

——— 1903. Les cellules interstitielles du testicule chez les animaux hibernants. *Arch. Ital. de biol.*, **40**, 323.

Goddard, T. R. 1920. Hypertrophy of the interstitial tissue of the testicle in man. *J. Anat.*, **54**, 173.

Goetsch, E. 1916. The influence of pituitary feeding upon growth and sexual development. An experimental study. *Bull. Johns Hopkins Hospital*, **27**, 29.

Goldmann, E. S. 1909. Die äussere und innere Sekretion des gesunden und kranken Organismus im Lichte der vitalen Farbung. *Beitr. z. klin. Chir.*, **64**, 192.

Goodale, H. D. 1919. Interstitial cells in the gonads of domestic fowl. *Anat. Rec.*, **16**, 247.

Goodpasture, E. W. 1918. An anatomical study of senescence in dogs, with especial reference to the relation of cellular changes of age to tumors. *J. Med. Research*, **38**, 127.

Gotta, H. 1923. Vitamine B et glandes sexuelles. *Compt. rend. Soc. de biol.*, **88**, 373.

Gould, H. N. 1923. Observations on the genital organs of a sex intergrade hog. *Anat. Rec.*, **26**, 241.

Griffiths, J. 1895. The effects upon the testes of ligature of the spermatic artery, spermatic veins, and both artery and veins. *J. Anat. and Physiol.*, **30**, 81.

Guyer, M. F. 1912. Modification in the testes of hybrids from the guinea and the common fowl. *J. of Morph.*, **23**, 45.

Hanes, F. M. 1911. The relations of the interstitial cells of Leydig to the production of an internal secretion by the mammalian testis. *J. Exp. Med.*, **13**, 338.

von Hansemann, D. 1895. Über die sogenannten Zwischenzellen des Hodens und deren Bedeutung bei pathologischen Veränderungen. *Arch. f. path. Anat. u. Physiol., u. f. klin. Med.*, **142**, 538.

——— 1896. Über die grossen Zwischenzellen der Hoden. *Arch. f. Anat. u. Physiol., Physiol. Abt.*, 176.

Harms, W. 1914. *Experimentelle Untersuchungen über die innere Sekretion der Keimdrüsen und deren Beziehung zum Gesamforganismus.* Jena: Gustav Fischer.

——— 1922. Keimdrüsen und Alterszustand. *Fortschr. d. Naturwissensch., Forsch.*, **77**, 189.

Harvey, R. J. 1875. Über die Zwischensubstanz des Hoden. *Centralbl. f. d. med. Wissensch.*, **13**, 497.

Hedinger, E. 1920. Über Wucherung der Leydigschen Zwischenzellen bei Chorionepitheliom des Hodens. *Ztschr. f. ang. Anat. u. Konstit.*, **7**, 55.

Herxheimer, G., and Hoffmann, K. F. 1908. Über die anatomischen Wirkungen der Röntgenstrahlen auf den Hoden. *Deutsch. med. Wchnschr.*, **34**, 1551.

Hofmeister, H. 1872. Untersuchungen über die Zwischensubstanz im Hoden der Säugethiere. *Sitzungsb. d. k. Akad. d. Wissensch., Math.-naturw. Classe*, Wien, **65**, Abt. 3, 77.

Hofstätter, R. 1923. Experimentelle Studie über die Einwirkung des Nicotins auf die Keimdrüsen und auf die Fortpflanzung. *Arch. f. path. Anat. u. Physiol. u. f. klin. Med.*, **244**, 183.

Hooker, D. R. 1922. The effects of X-ray upon reproduction in the rat. *Am. J. Hygiene*, **2**, 461.

——— 1925. The effect of exposure to Roentgen rays on reproduction in male rats. *Am. J. Roentgen.*, **14**, 327.

Hoskins, R. G. 1925. Studies on vigor. IV. The effect of testicle grafts on spontaneous activity. *Endocrinology*, **9**, 277.

Houlbert, G. 1919. Rôle des vitamines dans la croissance. *Paris méd.*, **33**, 473.

Houssay, F. 1907. Variations expérimentales. Études sur six générations de poules carnivores. *Arch. de zool. exp. et gén.*, **6**, 137.

Hüssy, P., and Wallart, J. 1915. Interstitielle Drüse und Röntgenkastration. *Ztschr. f. Geburtsh. u. Gynäk.*, **77**, 177.

Humphrey, R. R. 1921. The interstitial cells of the urodele testis. *Am. J. Anat.*, **29**, 213.

——— 1926. Effects of vasoligation in sheep and goat. *Anat. Rec.*, **33**, 41.

Iscovesco, H. 1913. Action physiologique d'un lipoïde (nBb) extrait du testicule. *Compt. rend. Soc. de biol.*, **75**, 445.

Ishibashi, M. 1919. Über di Zwischenzellen mit besonderer Berücksichtigung ihrer pathologischen Verhältnisse. *Mitt. a. d. med. Fak. d. k. Univ., Tokyo*, **22**, 39.

Jackson, C. M. 1925. *The effects of inanition and malnutrition upon growth and structure.* Philadelphia: P. Blakiston's Son and Company.

Jaffé, R. 1921. Pathologischanatomische Veränderungen der Keimdrüsen bei Konstitutionskrankheiten im besonderen bei der Pädatrophie. *Frankf. Ztschr. f. Path.*, **26**, 250. Also *Verhandl. d. deutsch. path. Ges.*, **18**, 204.

Jordan, H. E. 1911. The spermatogenesis of the opossum (Didelphys virginiana) with special reference to the accessory chromosome and the chondriosomes. *Arch. f. Zellforsch.*, **7**, 41.

Kasai, K. 1908. Über die Zwischenzellen des Hodens. *Arch. f. path. Anat. u. Physiol. u. f. klin. Med.*, **194**, 1.

Kaufmann, E. 1911. *Lehrbuch der speziellen pathologischen Anatomie.* G. Reimer, 2, 911.

Kennedy, W. P. 1926. Reproduction and diet in the rat. *Quart. J. Exper. Physiol.*, **16**, 281.

Keufsler, H. von. 1920. Über einige Fälle von Hermaphroditismus mit besonderer Berücksichtigung der Zwischenzellen. *Beitr. z. path. Anat. u. z. allg. Path.*, **67**, 416.

Kitahara, Y. 1923. Über die Entstehung der Zwischenzellen der Keimdrüsen des Menschen und der Säugetiere und über deren physiologische Bedeutung. *Arch. f. Entwcklngsmechn. d. Organ.*, **52**, 550.

Koch, K. 1910. Zwischenzellen und Hodenatrophie. *Arch. f. path. Anat. u. Physiol. u. f. Klin. Med.*, **202**, 376.

Kohn, A. 1920. Der Bauplan der Keimdrüsen. *Arch. f. Entwcklngsmechn. d. Organ.*, **47**, 95.

Kolmer, W., and Koppányi, T. 1923. Über die Entwicklung des Pleurodeleshodens und der in ihm auffallenden Zwischenzellenbildungen. *Ztschr. f. Anat. u. Entwcklngsgesch.*, **69**, 304.

Kolmer, W., and Scheminzky, F. 1922. Finden sich Zwischenzellen nur bei den höheren Wirbeltieren? *Arch. f. d. ges. Physiol.*, **194**, 352.

Kopeć, S. 1918. (Contribution to the study of the nuptial color of fishes.) *Sprawozdania Taw. Nauk. Warz. Rok*, 11 (cited by Blacher, 1926); also *Compt. rend. Soc. sc. de Varsovie*, **11**, 88 (cited by van Oordt, 1925).

Kostitch, A. 1921*a*. Sur la dissociation de la glande séminale et de la glande interstitielle déterminée par l'alcoolisme expérimental. Stérilité sans impuissance. *Compt. rend. Soc. de biol.*, **84**, 569.

——— 1921*b*. Sur l'involution du processus spermatogénétique provoquée par l'alcoolisme expérimental. *Ibid.*, **84**, 674.

Kostitch, A., and Tebebakovitch, A. 1926. Action de l'iode sur le testicule du rat blanc. *Compt. rend. Soc. de. biol.*, **95**, 986.

Kraus, E. J. 1921. Pankreas und Hypophyse. (Eine tierexperimentelle Studie.) *Beitr. z. patb. Anat. u. allg. Patb.*, **68**, 258.

Kronfeld, A. 1921. Zur Morphogenese des Zwischengewebes der Keimdrüsen, nach den Untersuchungen Guileras. *Arch. f. Frauenkunde*, **7**, 242.

Kuntz, A. 1919*a*. The innervation of the gonads in the dog. *Anat. Rec.*, **17**, 203.

——— 1919*b*. Experimental degeneration in the testis of the dog. *Ibid.*, **17**, 221.

——— 1920. Induced testicular degeneration and accompanying hypertrophy of the interstitial tissue. *Ibid.*, **18**, 237.

——— 1921. Degenerative changes in the seminal epithelium and associated hyperplasia of the interstitial tissue in the mammalian testis. *Endocrinology*, **5**, 190.

Kunze, A. 1922*a*. Das physiologische Vorkommen morphologisch darstellbarer lipoide in Hoden und Prostata mit besonderer Berücksichtigung der Haussäugestiere. *Arch. f. mikr. Anat.*, **96**, 387.

——— 1922*b*. Über Zwischenzellentumoren im Hoden des Hundes. *Arch. f. path. Anat. u. Physiol. u. f. klin. Med.*, **240**, 144.

Kyrle, J. 1911. Über die Regenerationsvorgänge im tierischen und menschlichen Hoden. *Sitzungsb. d. k. Akad. d. Wissensch., Math.-Naturw. Klasse*, Wien, **120**, Abt. 3, 3.

——— 1920. Über die Hypoplasie der Hoden im Jugendalter und ihre Bedeutung für das weitere Schicksal der Keimdrüsen. *Wien. klin. Wchnschr.*, **33**, No. 9, 185.

Kyrle, J., and Schopper, K. J. 1914. Untersuchungen über den Einfluss des Alkahols auf Leber und Hoden des Kaninchens. *Arch. f. path. Anat. u. f. klin. Med.*, **215**, 309.

Lahm, W. 1922. Zur Entwicklung der interstitiellen Drüse im Hoden und Ovarium. *Monatschr. f. Geburtsh. und Gynäk.*, **58**, 128.

Lehrt, E. 1904. Zur Kenntniss der Fetthaltigen Pigmente. *Arch. f. path. Anat. u. Physiol. u. klin. Med.*, **177**, 248.

Lenhossék, M. von. 1897. Beiträge zur Kenntniss der Zwischenzellen des Hodens. *Arch. f. Anat. u. Physiol., anat. Abt.* (*Arch. f. Anat. u. Entwcklngsgesch.*), Jahrg. 1897, 65.

Lenninger, W. 1923. Das Hodenzwischengewebe der Haussäugetiere. *Ztschr. f. Anat. u. Entwcklngsgesch.*, **68**, 230.

Lespinasse, V. D. 1922. The testicle as a gland of internal secretion. In *Endocrinology and Metabolism*. New York: D. Appleton and Company, **2**, 491.

Leupold, E. 1921. Cholesterinstoffwechsel und Spermiogenese. *Beit. z. path. Anat. u. z. allg. Path.*, **69**, 305.

Leydig, F. 1850. Zur Anatomie der männlichen Geschlechtsorgane und Analdrüsen der Säugetiere. *Ztschr. f. wiss. Zool.*, **2**, 1.

Lillie, F. R. 1917. The free-martin; a study of the action of sex hormones in the foetal life of cattle. *J. Exp. Zool.*, **23**, 371.

——— 1923. Supplementary notes on twins in cattle. *Biol. Bull.*, **44**, 47.

Lindsay, B., and Medes, G. 1926. Histological changes in the testis of the guinea-pig during scurvy and inanition. *Am. J. Anat.*, **37**, 213.

Lipschütz, A. 1923. Bemerkungen zur Arbeit von H. Stieve: "Neue Untersuchungen über die Zwischenzellen." *Anat. Anz.*, **56**, 564.

——— 1924. *The internal secretions of the sex glands.* Baltimore: Williams and Wilkins Company; also Cambridge: W. Heffer and Sons, Ltd. (The numerous publications of Lipschütz and his co-workers, as well as the literature generally, are listed and reviewed here.)

——— 1925*a*. Is there an antagonism between the male and the female sex-endocrine glands? *Endocrinology*, **9**, 109.

——— and co-workers. 1925*b*. Experimenteller Hermaphroditismus und der Antagonismus der Geschlechtsdrüsen. *Arch. f. d. ges. Physiol.*, I Mitteilung, **207**, 548; II Mitteilung, **207**, 563; III Mitteilung, **207**, 583; IV Mitteilung, **208**, 272; V Mitteilung, **208**, 293.

Loisel, G. 1902. Études sur le spermatogénèse chez le moineau domestique. *Jour. de l'anat. et de la physiol.*, **33**, 112.

——— 1904*a*. Les pigments élaborès par le testicule du poulet. *Compt. rend. Soc. de biol.*, **56**, 404.

——— 1904*b*. Sur l'origine et la double signification des cellules interstitielles du testicule. *Ibid.*, **56**, 448.

Lotz, A., and Jaffé, R. 1924. Die Hoden bei Allgemeinerkrankungen. *Ztschr. f. Konstit.*, **10**, 99.

Lubarsch, O. 1896. Über das Vorkommen von krystallinischer und krystalloiden Bildungen in dem Zellen des menschlichen Hodens. *Arch. f. path. Anat. u. Physiol. u. f. klin. Med.*, **145**, 316.

——— 1921. *Verhandl. d. deutsch. path. Ges.*, **18**, 210.

Lundgren, P. G. 1925. Zur Altersanatomie des Kaninchenhodens. Ein Beitrag zur Konstitutionsanatomie. *Ztschr. f. mikr.-anat. Forschr.*, **4**, 41.

McCarrison, R. 1921. *Studies in deficiency disease.* London: Frowde, Hodder and Stoughton.

Macht, D. I., and Teagarden, E. J. 1923. Rejuvenation experiments with vas ligation in rat. *J. Urol.*, **10**, 407.

Mann, F. C. 1916. The ductless glands and hibernation. *Am. J. Physiol.*, **41**, 173.

Maslowsky, W. 1923. (Cited by Stefko, 1924.)

Mason, K. E. 1925. A histological study of sterility in the albino rat due to a dietary deficiency. *Proc. Nat. Acad. Sc.*, **11**, 377.

——— 1926. Testicular degeneration in albino rats fed a purified food ration. *J. Exper. Zool.*, **45**, 159.

Massaglia, A. C. 1920. The internal secretion of the testis. *Endocrinology*, **4**, 547.

Mathieu, C. 1898. *De la cellule interstitielle du testicule et de ses produits de secrétion (cristalloïdes).* Thèse de Nancy.

Mattei, C. 1914. *Anatomie pathologique des glandes à secrétion interne dans l'athrepsie.* Thèse de Montpellier.

Mattill, H. A., and Carman, J. S. 1923. The degeneration of the testis of rats on a milk diet. *Proc. Soc. Exp. Biol.*, **20**, 420.

Mattill, H. A., and Clayton, M. M. 1926. Vitamin E and reproduction on synthetic and milk diets. *J. Biol. Chem.*, **68**, 665.

Maximow, A. 1899. Die histologischen Vorgänge bei der Heilung von Hodenverletzungen und die Regenerationsfähigkeit des Hodengewebes. *Beitr. z. path. Anat. u. z. allg. Path.*, **26**, 230.

Mazzetti, L. 1911. I caratteri sessuali secondari e le cellule interstiziali del testicolo. *Anat. Anz.*, **38**, 361.

Meyerstein, A. 1922. Anatomische Untersuchungen zur Frage der akzessorischen Nährstoffe. *Arch. f. path. Anat. u. Physiol. u. f. klin. Med.*, **239**, 350.

Mihalkowics, V. 1873. Beiträge zur Anatomie und Histologie des Hodens. *Ber. u. d. Verhandl. K. Sächs. Gesellsch. d. Wissensch.*, Math.-phys. Klasse (Leipzig), **25**, 217.

Mills, R. G. 1919. The pathological changes in the testes in epidemic pneumonia. *J. Exper. Med.*, **30**, 505.

Mita, G. 1914. Physiologische und pathologische Veranderungen der menschlichen Keimdruse von der Fetalen zur Pubertätszeit mit besonderer Beruchsichtigung der Entwickelung. *Beit. z. allg. Path. u. path. Anat.*, **58**, 554.

Monterosso, B. 1912. L'azione del digiuno e dell'estratto secco di tiroide sulla struttura dell'epitelio del tubo seminifero del topo. *Arch. de biol.*, **27**, 35.

Monterosso, B., and Schlatter, B. 1911. Sull'origine del grasso nei tubi seminifero del topo (*Mus decumanus var. alb.*). *Bull. Accad. Giornia Sc. nat. Catania*, Ser. **2**, Fasc. 15, 28.

Moore, C. R. 1919. On the physiological properties of the gonads as controllers of somatic and psychical characteristics. *J. Exper. Zool.*, **28**, 137.

——— 1923. On the relationship of the germinal epithelium to the position of the testis. *Anat. Rec.*, **25**, 142.

——— 1924*a*. Properties of the gonads as controllers of somatic and psychical characteristics. VI. Testicular reactions in experimental cryptorchidism. *Am. J. Anat.*, **34**, 269.

——— 1924*b*. Properties of the gonads as controllers of somatic and psychical characteristics. VIII. Heat application and testicular degeneration; the function of the scrotum. *Ibid.*, **34**, 337.

——— 1925. Sex determination and sex differentiation in birds and mammals. *Amer. Nat.*, **59**, 177.

——— 1926*a*. The activity of displaced testes and its bearing on the problem of the function of the scrotum. *Am. J. Physiol.*, **77**, 59.

——— 1926*b*. On the properties of the gonads as controllers of the somatic and psychical characteristics. IX. Testis graft reactions in different environments. *Am. J. Anat.*, **37**, 351.

——— 1926*c*. Scrotal replacement of experimental cryptorchid testes and the recovery of spermatogenic function (guinea-pig). *Biol. Bull.*, **51**, 112.

——— 1926*d*. The biology of the mammalian testis and scrotum. *Quart. Rev. of Biol.*, 1, 4.

Moore, C. R., and Oslund, R. M. 1924. Experiments on the sheep testes cryptorchidism, vasectomy and scrotal insulation. *Am. J. Physiol.*, **67**, 595.

Moore, C. R., and Quick, W. J. 1924. Properties of the gonads as controllers of somatic and psychical characteristics. VII. Vasectomy in the rabbit. *Am. J. Anat.*, **34**, 317.

Morgan, T. H. 1915-16. Demonstration of the appearance after castration of cock-feathering, in a hen-feathered cockerel. *Proc. Soc. Exp. Biol. and Med.*, **13**, 31.

Morgenstern, Z. 1923. Zur Frage des morphologischer Verhaltens des Hodens bei akuten Infektionskrankheiten. *Arch. f. path. Anat. u. Physiol. u. f. klin. Med.*, **245**, 229.

Mott, F. W. 1919. Normal and morbid conditions of the testis from birth to old age in one hundred asylum and hospital cases. *Brit. Med. J.*, **2**, 655, 698, 737.

Mottram, J. C., and Cramer, W. 1923. On the general effects of exposure to radium on metabolism and tumour growth in the rat and the special effects on testis and pituitary. *Quart. J. Exper. Physiol.*, **13**, 209.

Myers, B. D. 1915. Histological changes in testes following vasectomy. *Anat. Rec.*, **10**, 228.

Nemenov. 1916. Sur l'influence de la roentgenisation des testicules sur la prostate. *Arch. d. sc. biol.*, **19** (cited by Lipschütz, 1924, p. 154).

Nonidez, J. F. 1920. Studies on the gonads of the fowl. I. Hematopoietic processes in the gonads of embryos and mature birds. *Am. J. Anat.*, **28**, 81.

—— —— 1922. Studies on the gonads of the fowl. III. The origin of the so-called luteal cells in the testis of hen-feathered cock. *Ibid.*, **31**, 109.

—— —— 1924*a*. Studies on the gonads of the fowl. IV. The intertubular tissues of the testis in normal and hen-feathered cocks. *Ibid.*, **34**, 359.

—— —— 1924*b*. Studies on the gonads of the fowl. V. The effect of ligation of the vas deferens on the structure of the testis. *Ibid.*, **34**, 393.

Novaro, P. 1920. Tessuto testiculare et avitaminosi. *Gaz. degli Osp. Milano*), **41**, 424.

Nussbaum, M. 1880. Zur Differenzirung des Geschlechts im Thierreich. *Arch. f. mikr. Anat.*, **18**, 1–121 (particularly 85–96).

Odiorne, W. B., and Simmons, C. C. 1904. Undescended testicle. *Ann. Surg.*, **40**, 962.

Okuneff, N. 1922. Zur Morphologie der lipoiden Substanzen im Hungerzustande. *Beitr. z. path. Anat. u. z. allg. Path.*, **71**, 99.

—— 1923. Studien über Zellveränderungen im Hungerzustande (das Chondriom). *Arch. f. mikr. Anat.*, **97**, 187.

Oordt, G. J. van. 1924. Die Veränderungen des Hodens während des Auftretens der sekundären Geschlechtsmerkmale bei Fischen. I. *Gasterosteus pungitius L. Arch. f. mikr. Anat.*, **102**, 379.

—— 1925. The relation between the development of the secondary sex characters and the structure of the testis in the teleost *Xiphophorus Helleri Heckel. Brit. J. Exper. Biol.*, **3**, 43.

Oppermann, E., and Jaffé, R. 1924. Lipoiduntersuchungen im kindlichen Hoden. *Ztschr. f. Konstit.*, **10**, 111.

Osborne, T. B., and Mendel, L. B. 1918. Continuation and extension work on vegetable proteins. *Year Book of the Carnegie Institution of Washington*, **302**.

Oslund, R. 1924*a*. A study of vasectomy on rats and guinea-pigs. *Am. J. Physiol.*, **67**, 422.

—— 1924*b*. Vasectomy and rejuvenescence. *Science*, n. s., **60**, 419.

—— 1924*c*. Interstitial hypertrophy. *Am. J. Physiol.*, **69**, 589.

—— 1924*d*. Vasectomy on dogs. *Ibid.*, **70**, 111.

—— 1926*a*. Cryptorchid testis and testicular hormone production. *Ibid.*, **77**, 76.

—— 1926*b*. Ligation of vasa efferentia in rats. *Ibid.*, **77**, 83.

Parhon, C. I., and Parhon, C. 1922. Sur l'involution estiwale des caractères sexuels secondaires du plumage chez le canard mâle et sur les modifications parallèles du testicule chez le même animal. *Compt. rend. Soc. d. biol.*, **87**, 1227.

Parkes, A. S. 1925. The effects on fertility and the sex-ratio of sub-sterility exposures to X-rays. *Proc. Royal Soc. of London*, Series B, **98**, 415.

Parkes, A. S., and Drummond, J. C. 1925. The effect of vitamin B deficiency on reproduction. *Proc. Royal Soc. London*, Series B, **98**, 147.

—— 1926. The effects of fat-soluble vitamin A deficiency on reproduction in the rat. *Brit. J. Exper. Biol.*, **3**, 251.

Paul, C. B. 1906. On the influence of an excessive meat diet on the male reproductive organs. *Proc. Physiol. Soc. London, J. Physiol.*, **34**, XIV.

Pease, M. S. 1922. Note on Prof. T. H. Morgan's theory of hen feathering in cocks. *Proc. Cambridge Phil. Soc.*, **21**, Pt. 1, 22.

Pellegrini, G. 1925. Sulle modificazioni degli elementi interstiziali del testicolo negli animali ad attivita sessuale periodica. *Arch. ital. di anat. e di embriol.*, **22**, 550.

Pezard, A. 1918. *Le conditionnement physiologique des caractères sexuels secondaires chez les oiseaux*. Thèse. Paris: Édition du Bulletin Biologique de la France et de la Belgique.

——— 1919. Castration alimentaire chez les coqs soumis au régime carné exclusif. *Compt. rend. de l'Acad. d. Sc.*, Paris, **169**, 1177.

——— 1920. Secondary sexual characteristics and endocrinology. *Endocrinology*, **4**, 527.

——— 1922. La loi du "tout ou rien" et le gynandromorphisme chez les oiseaux. *J. de physiol. et de path. gén.*, **20**, 200, 495.

Pick, L. 1914. Über den wahren Hermaphroditismus des Menschen und der Säugetiere. *Arch. f. mikr. Anat.*, **84**, Abt. 2, 119.

Plato, J. 1896. Die interstitiellen Zellen des Hodens und ihre physiologische Bedeutung. *Arch. f. mikr. Anat.*, **48**, 280.

——— 1897. Zur Kenntniss der Anatomie und Physiologie der Geschlechtsorgane. *Ibid.*, **50**, 640.

Poll, H. 1920. Zwischenzellengeschwülste des Hodens bei Vogelmischlingen. *Beitr. z. path. Anat. u. z. allg. Path.*, **67**, 40.

Popoff, N. 1909. L'ovule mâle et le tissu interstitiel du testicule chez les animaux et chez l'homme. *Arch. de Biol.*, **24**, 433.

Portier, P. 1920*a*. Modifications du testicule des oiseaux sous l'influence de la carence. *Compt. rend. Acad. des Sc.*, Paris, **170**, 755.

——— 1920*b*. Régéneration du testicule chez le pigeon carencé. *Ibid.*, **170**, 1339.

Quick, W. J. 1926. Injection of testis material into the rat and its effects on spermatogenesis. *Am. J. Physiol.*, **77**, 51.

Rasmussen, A. T. 1917. Seasonal changes in the interstitial cells of the testis in the woodchuck (Marmota monax). *Am. J. Anat.*, **22**, 475.

——— 1918. Cyclic changes in the interstitial cells of the ovary and testis in the woodchuck (Marmota monax). *Endocrinology*, **2**, 353.

Regaud, C. 1900. Note sur le tissue conjonctif du testicule, chez le rat. *Compt. rend. Soc. de biol.*, **52**, 26.

——— 1901. Études sur la structure des tubes séminifères et sur les spermatogénèse chez les mammifères. *Arch. d'anat. mikr.*, **4**, 101.

Regaud, C., and Dubreuil, G. 1907. Action des rayons de Röntgen sur le testicule du lapin. 1. Conservation de la puissance virile et stérilization. *Ibid.*, **63**, 647.

——— 1908. Action des rayons de Röntgen sur le testicule des animaux impubères: immunité (relative) de l'epithélium séminal. *Ibid.*, **65**, 393.

Reinke, F. 1896. Beiträge zur Histologie des Menschen. 1. Über Krystalloidbildungen in den interstitiellen Zellen des menschlichen Hodens. *Arch. f. mikr. Anat.*, **47**, 34.

Reiss, P. 1923. Sur les caractères sexuels secondaires chez le lézard mâle. *Compt. rend. Soc. d. biol.*, **88**, 445.

——— 1923. Le cycle testiculaire du lézard. *Ibid.*, **88**, 447.

Remotti. 1923. Comportamento dei lipoidi nelle cellule di Sertoli e nel tessuto interstiziale del testicolo dei Selaci. *Atti. Acad. Peloritana* (cited by Pellegrini, 1925).

Ricardi, A. 1924. Variazioni quantitative absolute delle cellule interstiziali del testicolo nelle diverse età. *Atti. d. Soc. Lombarda di Sc. med. e. biol.*, **13** (cited by Lundgren, 1925).

Romies, B. 1921. Untersuchungen zur Verjügungshypothese Steinachs. *Münch. med. Wochenschr.*, **68**, 600.

Roxas, H. A. 1926. Gonad cross-transplantation in sebright and leghorn fowls. *Jour. Exper. Zool.*, **46**, 63.

Rubaschkin, W. 1912. Zur Lehre von der Keimbahn bei Säugetieren. Über die Entwicklung der Keimdrüsen. *Anat. Hefte*, **46**, 343.

Sainmont, G. 1906–7. Recherches relatives à l'organogenèse du testicule et de l'ovaire chez le chat. *Arch. de Biol.*, **22**, 71.

Saller, K. 1926. Untersuchungen über die männliche Keimdrüse der weissen Hausmaus. 1 Teil: Einleitung, allgemeine Versuchsanordnung und Mengen bestimmungen. *Ztschr. f. Anat. u. Entwcklngsgesch.*, **80**, 579.

Sand, K. 1921. Études experimentales sur les glandes sexuelles chez les mammifères. *J. de physiol. et path. gén.*, **19**, 305, 494, 515.

—— 1922. Vasoligature (epididymectomy employed ad mod. Steinach with a view to restitution in cases of senium and other states (impotency, depression)). *Acta chir. Scandin.*, **55**, 387.

—— 1923. Experiments on the endocrinology of the sexual glands. *Endocrinology*, **7**, 273.

—— 1926. *Handbuch der normalen und pathologischen Physiologie, etc.* Berlin: Julius Springer. **14**, Teil 1, 215, 251, 299, 344.

Schafer, E. S. 1926. *The endocrine organs.* Ed. 2, Pt. 2, London: Longmans, Green and Co.

Scheunig, F. 1923. Zur Frage von Steinachs F-Zellen. *Arch. f. Gynäk.*, **116**, 660.

Schinz, H. R., and Slotopolsky, B. 1924. Über die Wirkung der Röntgenstrahlen auf den in der Entwicklung begriffenen Hoden. *Arch. f. mikr. Anat.*, **102**, 363.

—— 1925. Der Röntgenhoden. *Ergebn. d. med., Strahlenforschung*, **1**, 445.

Schweizer, R. 1925. Eine neue Hypothese über die Bedeutung der Zwischenzellen der Testis. *Schweiz. med. Wochenschr.*, **55**, 665.

Sehrt, E. 1904. Zur Kenntnis der fetthaltigen Pigmente. *Arch. f. path. Anat. u. Physiol. u. f. klin. Med.*, **177**, 248.

Shattock, S. G., and Seligmann, C. G. 1904. Observations upon the acquirement of secondary sexual characters, indicating the formation of an internal secretion by the testicle. *Proc. Roy. Soc. London*, **73**, 49; and 1905. *Trans. Path. Soc. London*, **56**, 57.

Simmonds, M. 1909. Über die Einwirkung von Röntgenstrahlung auf der Hoden. *Fortschr. auf d. Gebiet d. Röntgenstrahlen*, **14**, 229.

—— 1921. Über das Verhalten des menschlichen Hodens bei narbigem Verschluss des Samenleiters. *Verhandl. d. deutsch. path. Ges.*, **18**, 201.

Simnitzky, W. S. 1926. Beiträge zur Frage über das Interstitialgewebe des Hodens als Resorptionsorgan (Testes bei Avitaminose B). *Arch. f. path. Anat. u. Physiol. u. f. klin. Med.*, **261**, 265.

Simonowitsch, I. J. 1899. (Über pathologisch-anatomischen Veränderungen der Hoden bei vollständigem und unvollständigem Hungern der Tiere und Auffütterung nach dem Hunger.) *Centralbl. f. path. Anat.*, **10**, 195. (Abstract of Russian literature on inanition by Muhlmann. Original, St. Petersburg dissertation, 1896.)

Siperstein, D. M. 1921. The effects of acute and chronic inanition upon the development and structure of the testis in the albino rat. *Anat. Rec.*, **20**, 355.

Slotopolsky, B., and Schinz, H. R. 1925*a*. Histologische Hodenbefunde bei Sexualverbrechern. *Arch. f. path. Anat. u. Physiol. u. f. klin. Med.*, **257**, 294.

—— 1925*b*. Histologisches zur Steinach-Unterbindung. *Ztschr. f. mikr.-anat. Forsch.*, **2**, 225.

Smith, L. W. 1919. Senile changes of the testis and prostate in dogs. *J. of Med. Research*, **40**, 31.

Smith, P. E. 1926. Ablation and transplantation of the hypophysis in the rat. *Anat. Rec.*, **32**, 221.

Sorg, K. 1924. Lipoiduntersuchungen am Rinderhoden. *Ztschr. f. Konstit.*, **10**, 67.

Spangaro, S. 1901. Über die histologischen Veranderungen des Hodens, Nebenhodens und Samenleiters von Geburt an bis zum Greisenalter. *Anat. Hefte*, **18**, 592.

Speidel, C. C. 1925. Studies of hyperthyroidism. I. Experimental hyperthyroidism and the germ glands in amphibians, with special reference to the primitive interstitial tissue. *Anat. Rec.*, **31**, 65.

Stefko, W. H. 1923. Der Einfluss des Hungerns auf das Wachstum und die gesatenphysiche Entwicklung der Kinder (im Zusammenhang mit anatomischen Veran derungen beim Hungern). *Ztschr. f. Konstit.*, **9**, 312.

——— 1924. Die Herzensänderungen beim Hungern im Zusammenhang mit seineä konditionellen Besonderheiten als eines Organs. *Ibid.*, **9**, 501.

Steinach, E. 1911. Umstimmung des Geschlechtscharakters bei Säugetieren durch Austausch der Pubertätsdrüsen. *Zentralbl. f. Physiol.*, **25**, 723.

——— 1912. Wilkürliche Umwandlung von Säugetier-Männchen in Tiere mit augeprägt weiblichen Geschlechtscharakteren und weiblicher Psyche. Eine Untersuchung über die Funktion und Bedeutung der Pubertätsdrüsen. *Arch. f. d. ges. Physiol.*, **144**, 71.

——— 1920. *Verjüngung durch experimentelle Neubelebung der alternden Pubertätsdrüse.* Berlin: J. Springer; also *Arch. Entwcklngsmech. d. Organ.*, **46**, 553.

Steinach, E., and Kammerer, P. 1920. Klima und Mannbarkeit. *Arch. f. Entwcklngsmechn. d. Organ.*, **46**, 391.

Stieve, H. 1921*a*. Entwickelung Bau und Bedeutung der Keimdrüsenzwischenzellen. *Ergebn. d. Anat. u. Entwcklngsgesch.*, **23**, 1. Also published in book form, Münich and Wiesbaden: J. F. Bergmann.

——— 1921*b*. Neue Untersuchungen über die Zwischenzellen. *Verhandl. d. anat. Gesellsch.*, **30**, 63 (Ergänzungsheft z. *Anat. Anz.*, 54).

——— 1922. Untersuchungen über die Wechselbeziehungen zwischen Gesamtkörper und Keimdrüsen. I. Mastversuche an männlichen Gänsen. *Arch. f. Entwcklngsmechn. d. Organ.*, **52**, 313.

——— 1923*a*. Vergleichend physiologisch-anatomische Beobachtungen über die Zwischenzellen des Hodens. *Arch. f. d. ges. Physiol.*, **200**, 470.

——— 1923*b*. Untersuchungen über die Wechselbeziehungen zwischen Gesamtkörper und Keimdrüsen. II. Beobachtung und Versuche an männlichen Hausmäusen und an männlichen Feldmäusen, zugleich ein weiterer Beitrag zur Zwischenzellenfrage. *Arch. f. mikr. Anat.*, **99**, 390.

——— 1925. *Ibid.* IV. Histologische Beobachtungen an den Hoden und Hebenhoden eines durch Unterbindung beider Nebenhoden "verjüngten" Hundes. *Ztschr. f. mikr.-anat. Forsch.*, **2**, 111.

——— 1926. *Ibid.* V. Weitere untersuchungen und Versuche an männlichen und weiblichen Gänsen sowie an Haushähnen. Ein Beitrag zum Einfluss der Haustierwerdung (Domestikation) auf die Geschlechtstätigkeit und der Vermehrung der Arten nebst weitern Beobachtungen über das Zwischengewebe. *Ibid.*, **5**, 463.

Swingle, W. W. 1926. The determination of sex in animals. *Physiol. Reviews*, **6**, 28.

Takahashi, N. 1922*a*. Untersuchungen über die tonisierenden und trophischen Funktionen des Sympathicus. *Arch. f. d. ges. Physiol.*, **193**, 322.

——— 1922*b*. Hodenatrophie nach Extirpation des abdominalen Grenzstranges. *Ibid.*, **196**, 237.

Tandler, J., and Gross, S. 1913. *Die biologischen Grundlagen der sekundären Geschlechtscharaktere.* Berlin: J. Springer.

Thaler, H. A. 1904. Über das Vorkommen von Fett und Kristallen im menschlichen Testikel unter normalen und pathologischen Verhältnissen. *Beit. z. path. Anat. u. z. allg. Path.*, **36**, 529.

Thorek, M. 1924. *The human testis.* Philadelphia and London: J. B. Lippincott Company.

Tiedje. 1921. Unterbindungsbefunde am Hoden unter besonderer Berücksichtigung der Pubertätsdrüsenfrage. *Verhandl. d. deutsch. path. Ges.*, **18**, 200.

Traina, R. 1904. Über das Verhalten des Fettes und der Zellgranula bei chronischem Marasmus und akuten Hungerzuständen. *Beitr. z. path. Anat. u. z. allg. Path.*, **35**, 1.

Tsuji, K. 1920. On the function of the thyroid gland with special reference to the effect of variations of diet upon it. *Acata Scholae Med. Univ. Imp. Kioto*, **3**, 713.

Uffreduzzi, O. 1910. Liposomi e lipoidi della cellula interstiziale. *Gior. d. r. Accad. d. med. di Torino*, 4 s., **16**, 123 (see also *ibid.*, 36).

Villemin, F. 1906*a*. Rayons X et activité génitale. *Compt. rend. de l'Acad. Sc.*, Paris, **142**, 723.

——— 1906*b*. Sur la régénération de la glande séminale après destruction par les rayons X. *Compt. rend. Soc. de biol.*, **60**, 1076.

Voinov, D. 1905. Sur le rôle probable de la glande interstitielle. *Compt. rend. Soc. de biol.*, **58**, 414.

Wagner, K. 1923. Zur Zytologie der Zwischenzellen des Hodens. *Anat. Anz.*, **56**, 550.

——— 1925. Sind die Zwischenzellen des Säugetierhodens Drüsenzellen? Ein Beitrag zur Zytologie und Zytogenese. *Biologia Generalis*, **1**, 22.

Walker, K. M. 1924. The internal secretion of the testis. *Lancet*, **1**, 16.

Wangensteen, O. H. 1927. The undescended testis. An experimental and clinical study. *Arch. Surg.*, **14**, 663.

Warwick, B. L. 1925. The effect of vasectomy on swine. *Anat. Rec.*, **31**, 19.

Watson, A. 1919. A study of the seasonal changes in avian testis. *J. Physiol.*, **53**, 86.

Weichselbaum, A. 1910. Über Veränderungen der Hoden bei chronischem Alkoholismus. *Verhandl. d. deutsch. path. Gesellsch.*, **14**, 234.

Wheelon, H. 1922. Physiology, physiological chemistry and experimental pathology of the testis. In *Endocrinology and Metabolism.* New York: D. Appleton and Company, **2**, 431.

Whitehead, R. H. 1904. The embryonic development of the interstitial cells of Leydig. *Am. J. Anat.*, **3**, 167.

——— 1905. Studies of the interstitial cells of Leydig. No. 2. Their post-embryonic development in the pig. *Ibid.*, **4**, 193.

——— 1908*a*. A peculiar case of cryptorchism and its bearing upon the problem of the function of the interstitial cells of the testis. *Anat. Rec.*, **2**, 177.

——— 1908*b*. Studies of the interstitial cells of Leydig. No. 3. Histology. *Ibid.*, **1**, 213.

——— 1912*a*. On the chemical nature of certain granules in the interstitial cells of the testis. *Am. Jour. Anat.*, **14**, 63.

——— 1912*b*. A microchemical study of the fatty bodies in the interstitial cells of the testis. *Anat. Rec.*, **6**, 65.

Willier, B. H. 1921. The structures and homologies of the free-martin gonads. *J. Exper. Zool.*, **33**, 63.

Winiwarter, H. von. 1912. Observations cytologiques sur les cellules interstitielles du testicle humain. *Anat. Anz.*, **41**, 309.

Yamakawa, H. 1925. The action of X-rays against the testicles of the albino rat, together with the vital staining of interstitial cells. *J. Toyko Med. Soc.*, **39** (abstract in *Endocrinology*, **10**, 75).

Yamasaki, Y. 1923. Experimentelle Untersuchungen über den Einfluss des Vitamin- oder Zellsalzmangels auf die Entwicklung von Spermatozoen und Eiern. *Arch. f. path. Anat. u. Physiol. u. f. klin. Med.*, **245**, 513.

Section XXXVI

THE MALE GERM CELLS

CONTENTS

SECTION XXXVI

PAGE

I. ORIGIN AND EARLY HISTORY OF GERM CELLS 1259
1. Invertebrates. 1260
2. Vertebrates. 1262
3. Origin of female germ cells in mammals . . . 1268
II. PRODUCTS OF EARLY GERM CELLS 1270
III. MATURATION 1270
1. Chromosomes. 1273
Chromosome behavior in Diptera—Outline of growth stages.
2. Chromosomes of man and other mammals 1282
3. Cytoplasmic constituents. 1282
Centrosomes, Golgi bodies and idiozome material—Mitochondria.
4. Sperm formation 1287
Development of spermatid—General sketch—Sperm formation in opossum.
IV. MORPHOLOGY OF SPERMATOZOA 1292
1. Flagellate sperms 1293
V. PATHOLOGICAL AND EXPERIMENTAL MODIFICATION OF GERM CELLS 1294
1. Cryptorchidism, vasectomy, vasotomy, transplantation, etc. 1294
2. Degeneration following dietary deficiencies, or treatment with alcohol, roentgen rays, etc.. 1296
3. Changes involved in degeneration 1296
VI. BIBLIOGRAPHY. 1297

Section XXXVI

THE MALE GERM CELLS

CHARLES W. METZ

THE subject of the present section embraces such a wide field, and one in which so many different lines of work have been carried out, that any brief treatment of it requires a radical limitation of the topics considered. The following account, therefore, is definitely restricted in scope. In conformity with the purpose of the book as a whole, emphasis is laid on conditions in mammals, and some parts of the account are restricted entirely to this field, although it has not seemed advisable to make such a restriction throughout. To give even a summary of certain topics has required the entire elimination of others. This has necessitated the omission of practically all special cases, many of which are of great interest but require a disproportionate amount of space for their discussion. For instance, it has not been possible to include a treatment of conditions in hybrids or in gynandromorphs, hermaphrodites, etc., much less those in the numerous exceptional cases presented by certain species or groups, such as the Hymenoptera, the aphids and phylloxerans, certain nematode worms and others.

For all such features, as well as for an adequate treatment of any of the major topics, the reader should consult other works. Foremost among the latter is Wilson's recent treatise on the cell (Wilson, 1925*a*), of which a large proportion is devoted to the germ cells. Various less extensive accounts of special topics will also be found in other recent books. Among these may be mentioned the chapters dealing with the germ cells and maturation phenomena in Sharp's "Cytology" (Ed. 2, 1926); those on germ cells and on sex determination in Doncaster's "Cytology" (1920); the sections on meiosis, the germ track and the sex chromosomes in Agar's "Cytology" (1920); and the chapter on the chromosome theory of heredity in "General Cytology" (1924).

Since the writer's work has been concerned more with the nuclear than with the cytoplasmic constituents of the germ cells, the reader is urged especially to consult other reviews of the latter subject. Such reviews are included in the above-mentioned books and also in the papers cited below (p. 1282).

I. ORIGIN AND EARLY HISTORY OF THE GERM CELLS

From the time of Weismann's classical studies, and even before, students of cytology and heredity have been concerned with the relation between the germ cells on the one hand, and the somatic or body cells on the other. Both the observations and the speculative considerations of Weismann and his supporters tended to draw a sharp line of distinction between the two, and

to indicate that whereas the germ cells retained all the potentialities of reproducing the organism, the somatic cells distributed these among themselves by a series of qualitative ontogenetic divisions, such that ultimately no one cell possessed a full set. The work of later observers has tended to break down this distinction to a considerable extent, and has shown that in its original form the view is untenable; but even to-day it is not certain as to how great or how little an actual difference exists between soma and germ.

1. *Invertebrates:*

In some animals the cells which are to give rise to germ cells are distinguishable even in the early cleavage stages of the egg. Such cells—known as "stem cells"—are characterized by their position or by the possession of a visible material or structure known variously as the "germ cell determinant," "Keimbahnplasma," etc. A variety of such cells has been described in different organisms. For instance, to cite a few of the classical examples, in the nematode worm, *Ascaris megalocephala*, in flies of the genus *Miastor* and in the crustacean *Cyclops fuscus*, one cell may be identified as the original stem cell. In other forms a group of cells is thus identified, usually by its position at one pole of the developing egg. Examples of the latter are seen in various insects (e.g., certain beetles, flies and Hymenoptera) in which the stem cells appear at one pole, easily distinguishable from the other cells (Figs. 581 to 584).

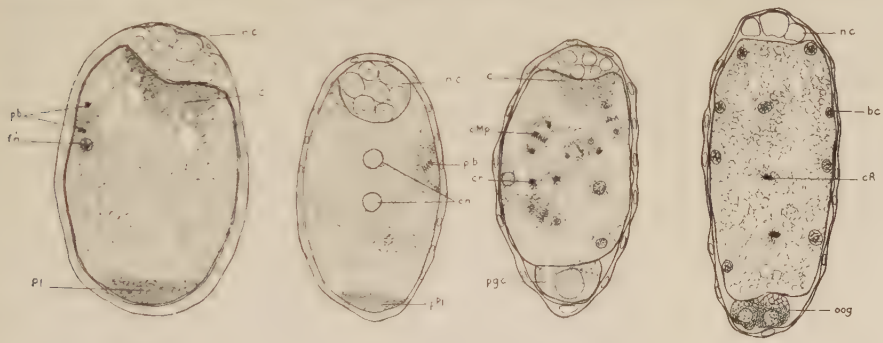
An account of such cases, together with a review of the whole subject, is given by Hegner (1914*a*, *b*). For present purposes attention may be confined to two features: (1) the structure and history of the stem cells, and (2) the phenomenon of "chromatin diminution," which is sometimes exhibited by the other cells, but not by the stem cells.

Both of these features are illustrated by the classical case of *Miastor* (a genus of pedogenetic flies), first described by Kahle (1908) and later by other workers, notably Hegner (1914*a*). The ripe egg of such a fly (conditions agree in both European and American species) is differentiated into regions as shown in Figure 581. At one end is a group of nurse cells (nc); in the center is the general cytoplasm (c), yolk, etc., together with the nucleus (fn); and at the other end is a granular mass of cytoplasm, the "polar plasm" (pl).

The latter is the "germ cell determinant," subsequently incorporated into the primordial germ cells. Figures 579 to 581 show the later history of such an egg during cleavage. At the third cleavage division three of the four nuclei (cell walls are not present) undergo a process of chromatin diminution in which considerable chromatin (cMp in Fig. 580) is given off from the chromosomes and left in the cytoplasm where it is long visible in the form of dark masses (cr). The descendants of these nuclei (ultimately cells), which may undergo further chromatin diminution, give rise only to somatic

tissue. The fourth nucleus, however, divides without any loss of chromatin. One of the two resulting nuclei passes into the region of the "polar plasm" at the end of the egg, after which a cell wall appears, enclosing the nucleus and polar plasm, and giving rise to a separate cell (Fig. 580 pgc). This is the primordial germ cell, from which come eventually all the germ cells of the animal.

The process of germ cell formation here has been followed step by step, apparently leaving no question as to the main facts. Furthermore, individual features, both as to the chromatin diminution process and the inclusion of the "germ cell determinant" in the primordial germ cells, are shown even more clearly in other organisms. The former, for instance, is illustrated



578 579 580 581

FIGS. 578–581.—Eggs of the fly Miastor, showing early differentiation of germ cells and the phenomenon of chromatin diminution. (After Hegner.) 1. Mature egg showing polar plasm (Pl). 2. Egg during cleavage showing cleavage nuclei (cn). 3. Later stage showing primordial germ cell (pgc) with the polar plasm incorporated in it, and showing elimination of chromatin (cMp and cr) by somatic nuclei. 4. Later stage showing multiplication of germ cells (oog) and of somatic cells (bc) and showing remains of eliminated chromatin.

in detail by the well-known case of *Ascaris* (Boveri, 1887, 1892) in which the fertilized egg contains only two chromosomes. At the second cleavage one cell undergoes no chromatin diminution; this is the ancestor of all the germ cells. In the other, the two chromosomes break up into segments, of which only the small median ones divide in the ordinary fashion, the large terminal ones being cast off. This cell gives rise only to somatic cells.

There is no reason, therefore, to doubt the fact that in *Miastor* the germ cells may be distinguished from the somatic cells by at least two characteristics —the possession of the full chromatin content, and the possession of the "polar plasm" or "germ cell determinant."

At first it was inferred that cases involving such a chromatin diminution afforded visible evidence of a loss of germ plasm from the somatic cells. But this view presupposes that the germ plasm is coextensive with chromatin—or, in other words, that all chromatin contains hereditary "genes" or "factors"—which is not certain. Indeed, in the writer's opinion, recent

LANE LIBRARY, STANFORD UNIVERSITY

genetic and cytological evidence* supports the view that although the genes are in the chromosomes, they do not necessarily constitute the entire substance of these bodies. If so, it is possible to view the above chromatin diminution process as a developmental phenomenon—an elimination of surplus chromatin—having nothing to do with the elimination of germ plasm.

This should not, however, be made to obscure the fact that in many forms the germ cells are set apart at an early embryonic stage and subsequently lead a semi-independent existence during the development of the organism.

2. *Vertebrates:*

When we turn to the vertebrates we find conditions materially different. So far as known to the writer, neither "germ cell determinants" nor the process of chromatin diminution has ever been observed here. In fact, it appears to be generally agreed that there is no visible morphological difference between germ cells and soma during cleavage. The germ cells are first distinguishable at a later period. But as to just when this distinction appears, and as to the actual history of the cells involved, there is wide divergence of opinion.

According to the early view of Waldeyer (1870) supported by other observers down to the present time, the germ cells are proliferated by the germinal epithelium of the gonads at a relatively late embryonic stage. According to others they are distinguishable by characteristics of size or structure at the time the germ layers are formed in the early embryo. The latter view identifies these "primordial" germ cells or stem cells, with those seen in many invertebrates, and thus brings the vertebrates into harmony with the long prevalent germ plasm theory. Partly, no doubt, as a result of such unification, this view has been widely accepted.

Recently, however, a marked tendency has been shown to return to the older view, or to a modification of it. Especially is this true as regards conditions in the mammals. With the controversy at its height, as it is at present, and with each of the major groups of vertebrates involved, we cannot hope to outline a generally accepted view, or even adequately to summarize the conflicting ones. At best we can only review a few of the main points at issue and cite illustrations from some of the most recent papers—to which the reader is referred for extended literature lists.

As intimated above, most of the divergent views may be separated broadly into two groups—the one considering the germ line as a definite lineal descent of germ cells distinct from the soma; the other seeing no such

* Such, e.g., as that indicating the presence of relatively few genes in the large Y-chromosome of *Drosophila* (Stern, 1926).

separation between germ line and soma, and even considering the germ cells as of somatic origin.

The former view may be stated somewhat as follows: The primordial germ cells arise from the endoderm or mesoderm (varying apparently with the organism) as morphologically distinct cells, which later migrate from their points of origin to the position of the germ glands, and ultimately give rise to the definitive germ cells. So many observers have agreed upon the main features here as to leave little doubt that some sort of segregation occurs. But a difficulty is introduced by the fact, now well established, that in several cases from widely separated groups the primordial germ cells tend to undergo degeneration in the embryo and that in some cases the definitive germ cells appear to arise independently at a later stage.

This has focussed attention on the stage at which degeneration occurs and on the question as to whether all the primordial germ cells degenerate, or part of them remain to give rise to the definitive germ cells. Numerous papers bearing on this and allied questions have appeared recently—among which may be cited those of Edgar Allen (1923), Beccari (1924), Firket (1920), Foley (1926), Gatenby (1924), Geiser (1924), Hargitt (1924, 1925, 1926) and Swingle (1921, 1926).

As representative of the view outlined above, it will be convenient to consider the recent work of Swingle on the bullfrog, especially in view of the fact that conditions in the frogs are themselves of particular interest. The earlier work of Allen (1907) traced "the history of the germ cells of anurans back to their origin in the entoderm where they are first distinguishable from other yolk-laden entodermal cells as a ridge just dorsal to the enteron and ventral to the aorta, separating the two lateral mesodermal plates. King (1908), Kuschakewitch (1910), Witschi (1914) and Swingle (1921) have confirmed Allen's observations." (Swingle, 1926, pp. 458 to 459.)

The later history of these cells is reviewed by Swingle somewhat as follows: As the gonads develop they are for a time indifferent, then distinguishable as ovaries or testes. Examples of the indifferent stage is shown in Figures 582 to 584, and successive stages in early testis development in Figures 585 and 586. The primordial germ cells are distinguishable here by their large size and their polymorphic nuclei.

In frog races which undergo ordinary direct development the testes differentiate directly from the stage shown in Figures 585 and 586 without any appreciable degeneration of the primordial germ cells (Witschi, Swingle). A later stage in such a testis is shown in Figure 587. "In such strains there can be no question but that the spermatozoa of the newly metamorphosed frog have been derived from the lineal descendants of the primordial germ cells" (Swingle, 1926, p. 502).

In other races of the same species, however, an indirect development occurs, involving an extensive degeneration of primordial germ cells. Here "progonads" are formed in male larvae, which differ in shape and structure from the definitive testes. Successive stages in the development of such an organ are shown in Figures 588 and 589 (compare with Figure 587). In the progonad the germ cells are collected in cysts and most of them undergo typical maturation stages (Fig. 588 a–c) up to the metaphase of the first spermatocyte division, as if they were going to mature and form spermatozoa in the larvae. But after reaching the metaphase they degenerate and disappear. Coincidently a few, which have remained undifferentiated, appear in the interior of the gonad (Fig. 589 rs)

PLATE I

FIGS. 582–587.—Origin and early history of germ cells in the bullfrog, *Rana catesbeiana*. (After Swingle.) Grateful acknowledgement is made to the Wistar Institute for supplying the cuts of these figures from the Journal of Morphology.

FIG. 582. Section through gonad of a 30 mm. tadpole after the appearance of the primary genital cavity (pc).

FIG. 583.—Section through a similar gonad in the region between the sex cords (represented by small dark cells in Fig. 582) showing large primary genital cavity (pc) filled with connective tissue.

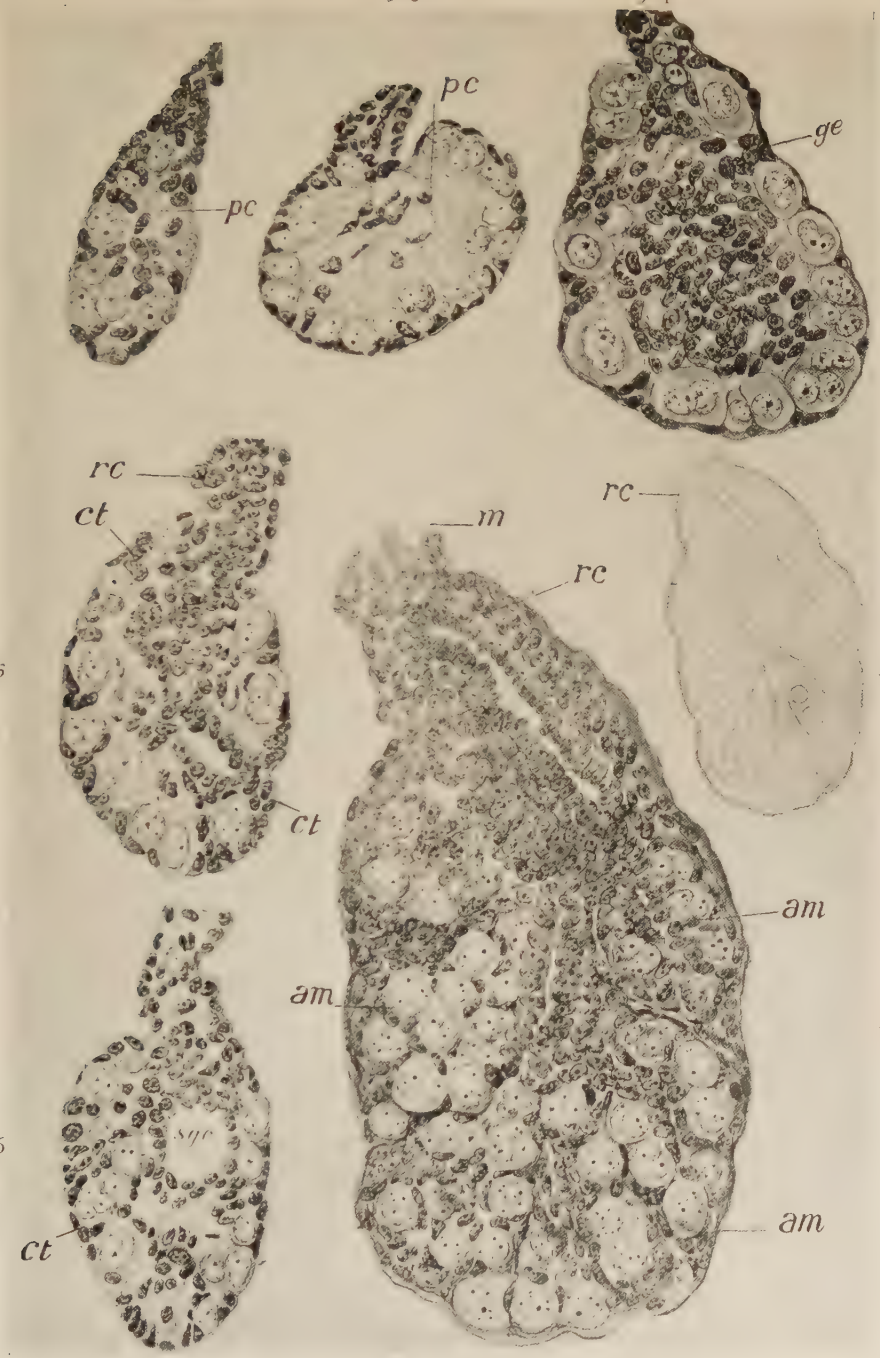
FIG. 584.—Section through 40 mm. gonad showing the maximum development of the morphologically undifferentiated germ gland. Germinal epithelium (ge). Large cells are germ cells; small ones sex cord elements.

FIG. 585.—Section showing first indication of differentiation into a testis—sex cord (rc) and its cells (ct) beginning to surround germ cells.

FIG. 586.—Somewhat more advanced stage of differentiation showing secondary genital cavity (sgc).

FIG. 587. —A, Testis showing the nest of germ cells (anlagen of testis ampullae (am) and mesentery (m). Structure shown schematically in B.

PLATE I


582
583
584
pc
pc
ge
rc
ct
m
rc
rc
585
587b
ct
ct
am
am
587a
586
sgc
ct
am

surrounded by the sex cords. These multiply and the definitive testis develops in the form of a core within the progonad as the cells of the latter degenerate. According to Swingle* who has traced "every stage in the origin, migration and development of these cells and their progeny up to the formation of spermatozoa" (1926, p. 501) –degeneration here only removes part of the primordial germ cells, and the remainder give rise to the definitive germ cells, with no break in the continuity of the germ line; so that in this regard there is no distinction between the different races, or types of development.*

This view agrees in its general aspects with that of Beccari (1924) on the toad, Swift (1915) on the fowl, Okkelberg (1921) on the brook lamprey, Allen (1906) and Jordan (1917) on the turtle and Rubaschkin (1912) on the guinea pig—to mention only a few examples. In the guinea pig the last-named author finds the germ cells readily distinguishable from the somatic cells by differences in their mitochondria. During cleavage all the cells have granular mitochondria, but when the germ layers are formed the mitochondria in the somatic cells become thread-like (Figs. 591 and 592) while those in the germ cells retain their granular form (Figs. 590 and 592). These conditions persist, so that the germ cells may thus be distinguished up to the final spermatogonial and oogonial stages (cf. also Woodger, 1925, for distinction based on Golgi bodies).

In contrast to such a conception is that of Hargitt (loc. cit.). Working on another amphibian (the salamander, *Diemyctylus*) and on the rat, he has come to the conclusion that, at least in the latter, there is no evidence that the germ cells are a part of a definite germ line. This view is based upon two contentions: (1) that no true primordial germ cells exist; and (2) that the so-called primordial germ cells of later stages degenerate without giving rise to the definitive germ cells—the latter arising from cells of the germinal epithelium which is essentially somatic tissue continuous with the peritoneal epithelium. On the first point Hargitt is in agreement with B. M. Allen (1904) on the pig and rabbit, and Simkins (1923) on the mouse.

According to Hargitt, "The large cells ('primordial germ cells') found outside the genital region, in different parts of the body, are not germ cells and have nothing to do with the formation of the gonad. Therefore, it is no longer proper to speak of a distinction between the germ cells and the body cells previous to the time when the gonad differentiates from the tissues of the embryo." (Hargitt, 1926, p. 278.)

Concerning the second point, Hargitt believes that the degeneration of the "large cells" (primordial germ cells of other workers) is really complete (cf. also Firket, 1920) but that, in the rat, only a short stage intervenes between their disappearance and the development of similar-looking cells from the germinal epithelium. Thus the critical stage is easily overlooked, which serves to explain the discrepancy in the various accounts.

* "The residual spermatogonia of the progonad lobules are the lineal descendants of the primordial sex cells of the embryo, and in turn are the mother cells from which are derived the germinal elements of the definitive testis . . . the 'Keimbahn' is continuous in this anuran." (Swingle, 1926, pp. 501 to 502.)

That Hargitt considers the general features comparable in different groups of vertebrates is clear from his discussion of the subject.*

To the writer it appears too early to generalize on this question—an opinion which is shared by some of the authors cited above.† But it is significant, for present purposes, that whatever view we take we must recognize a strong swing away from the general application of the Weismannian doctrine of an early and complete segregation of the germ cells.

The evidence is strong that some, at least, of the definitive germ cells in mammals arise from cells other than the "primordial germ cells"—and once this is admitted, the idea of an early segregation of the latter loses much of its significance. It is noteworthy, and probably not entirely a matter of coincidence, that this shift has been contemporaneous with the modern development of the chromosome theory of heredity, studies on tissue cultures, and experimental embryology, which have alike tended to remove the distinction between germ cells and soma and to regard all, save perhaps the most highly specialized, cells as possessing most of the inherited potentialities of germ cells, even though they cannot be made to function as such.

The distinction between germ cells and soma, then, may be analogous to that between the respective embryonic germ layers, the cells of which were formerly regarded as irreversibly differentiated at a very early stage, but have recently been shown by Spemann and Mangold to be equipotent and interchangeable until considerably later (cf. Mangold, 1925).

3. *Origin of female germ cells in mammals:*

In the preceding discussion attention has been focussed on the development of the testis, without regard to the ovary. And in most groups of animals little fundamental difference has been observed between the two up to the stage of maturation. In mammals, however, it has commonly been believed‡ that whereas the spermatocytes are proliferated from the spermatogonia repeatedly or continuously throughout the reproductive life of the animal, the definitive ova are all differentitated at an early embryonic stage, when the ovary is originally formed, and then lie quiescent until puberty—many years in some species. The recent work of Allen (1923)

* E.g., " . . . the problem of germ cell origin in mammals is similar to that of the Amphibia– it is not a question of constituents of egg protoplasm, but one of cellular differentiation from the embryo" (1925, p. 541). "An analysis of the argument for the specific character of the germ cells and their continuity from the egg shows that it is based largely upon assumption and not upon substantial observations. Germ cell origin is a question of cellular differentiation and not of early ontogenetic segregation" (1925, p. 542).

† Cf. Swingle, 1926, p. 502. Also Gatenby, 1924, p. 2.

‡ Cf. Allen, 1923, for literature.

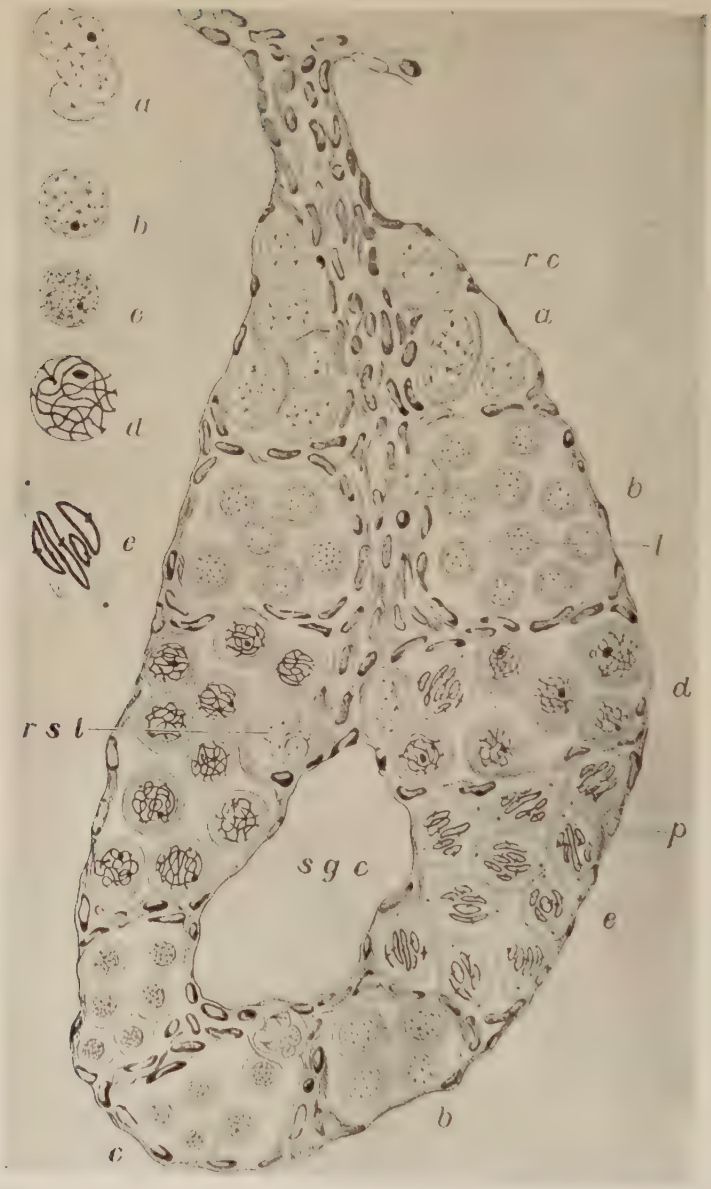


FIG. 588.—Section through testis undergoing indirect type of development. Progonad lobules (l) with cells in maturation stages (represented individually in a–e) and lobules of residual spermatogonia (rsl) and peritoneum (p).

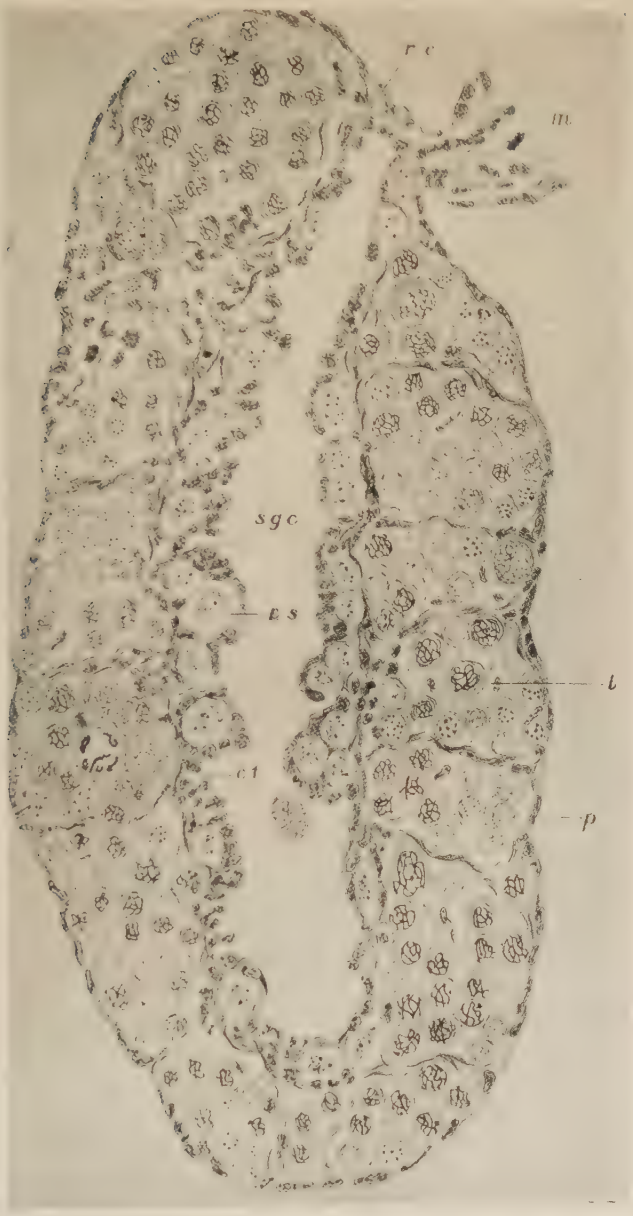


FIG. 589.—Later stage in indirect testis development, showing the appearance of the secondary genital cavity (sgc) in center. Projecting into this are the sex cords (small dark cells) enclosing the residual spermatogonia (rs) which multiply and give rise to the ultimate germ cells after the embryonic germ cells in the progonad lobules degenerate.

building upon that of Kingery and others, appears to show that this is not the case in the mouse, and hence probably not in other mammals.

Allen finds that the original ova degenerate and that the definitive ova arise by active division of cells in the germinal epithelium.* These findings appear to bring oogenesis into harmony with spermatogenesis in mammals and also into harmony with oogenesis in other vertebrates such as fishes, amphibians and reptiles.

II. PRODUCTS OF THE EARLY GERM CELLS

In addition to the ultimate gametes (and to degenerate germ cells where such are produced), the original germ cells may give rise to other cells, usually of a nutritive nature. Conditions differ widely in this respect. In many insects, such as most Diptera thus far studied, all the cells of the testis appear ultimately to give rise to spermatozoa. In others, one or more large nurse cells are found in the apical (spermatogonial) region of the testis, where they almost certainly serve to nourish the spermatogonia. Apparently these cells and the surrounding spermatogonia have a common origin. In mammals nurse cells are present in large numbers. These are the well-known Sertoli cells.

Perhaps the most extreme form of differentiation is that found in certain hermaphroditic animals where not only spermatozoa, but eggs, and at least two kinds of nurse cells are produced in the same gonad. Such a condition is described, for example, in the pulmonate mollusc *Testacella*, by Gatenby (1919).

III. MATURATION

Up to the time of maturation the germ cells undergo relatively little differentiation. The early spermatogonia may differ slightly from the late, and sometimes the last two generations can be identified individually; but the differences are largely those of size and form and need not concern us here. In general, the important stages are those of maturation, during which the relatively generalized spermatogonia give rise to the highly specialized spermatozoa. The changes taking place here appear to involve all the constituents of the cell, and are of interest alike to the morphologist, physiologist and student of heredity.

Too little is known of the physiology of this period to justify its discussion here; consequently attention will be confined to the morphological changes involved and to their bearing on the phenomena of inheritance.

* "A cyclical proliferation of the germinal epithelium gives rise to a new addition of young ova to the cortex of the adult ovary at each normal oestrous period" (Allen, 1923, p. 467). Contrast the views of Robinson (1918) and Arai (1920).

FIGS. 590–592.—Differentiation of germ cells in guinea pig. Fig. 590. Primordial germ cell—mitochondria granular. Fig. 591. Somatic (mesenchyme) cell from same embryo (9 mm.)—mitochondria filamentous. Fig. 592. Cross section of a testicular tubule of a 3.6 cm. embryo. Sp, spermatogonia; Ez. epithelial cells; Iz, interstitial cells; Bz, connective tissue. (After Rubaschkin.)

The latter feature, however, will be considered only in a general way, since it is treated fully in the preceding volume ("General Cytology," last two sections).

In its broader aspects the maturation of the sperm is like that of the egg —the final spermatogonium typically undergoes a period of growth, followed by two maturation divisions, during which the number of chromosomes is reduced by half. Two well-marked distinctions are usually evident, however: first, the spermatocyte only grows to a fraction of the size of the oocyte; and second, the primary spermatocyte, instead of producing three polar bodies and only one gamete, gives rise to four similar cells, all of which become gametes (spermatozoa).

Being a generalized cell the final spermatogonium, or primary spermatocyte at the beginning of the growth period, possesses no special peculiarities. Like most of the other cells of the body it contains the ordinary cellular components—chromosomes, nucleoli, centrosomes, mitochondria, Golgi bodies and other formed elements, together with the visibly undifferentiated cytoplasm. From the present standpoint, interest in spermatogenesis centers largely in the history of these components during the maturation stages, and their ultimate fate in the sperm. Probably no other field of cytology has received the attention accorded to this in the last fifty years; and perhaps in no other field have so many diverse conditions been revealed or so many divergent interpretations and opinions been expressed. Especially is this true as regards the chromosomes and their rôle in heredity. Fortunately, however, a sufficiently general agreement has now been reached on the latter subject, at least, to permit of a summary treatment here.

Both theoretically and practically the components of the germ cells fall into two categories —the nuclear and the cytoplasmic. The former have been shown to be primarily concerned with the processes of inheritance. The latter, although by no means negligible from this standpoint, have not been shown to be, and probably are not, agents in hereditary transmission comparable with the former. For this reason, as well as from the fact that different technical methods are required for their study, the nuclear and cytoplasmic constituents have seldom been considered together most recent studies having dealt primarily with the one or the other.

Leaving out of account the membranes, ground substance (plasma) inclusions, etc., which require no special treatment here, we may classify the cellular components as follows:

Nuclear. Chromosomes and nucleoli (plasmosomes).

Cytoplasmic. Centrioles, mitochondria and Golgi bodies (including the idiosome material).

Another, and perhaps more useful classification is one which will separate them on the basis of genetic continuity, and thus aid in interpreting

their behavior and in estimating their relative importance. For instance, we may distinguish them thus:

Self-perpetuating. Chromosomes.

Probably Self-perpetuating. Centrosomes.

Possibly Self-perpetuating. Mitochondria and Golgi bodies.

Probably Not Self-perpetuating. Nucleoli (excepting "chromosome nucleoli").

This classification permits a summary disposal of the nucleoli (plasmosomes). Since these structures give the appearance, at least, of arising de novo and since their physiological function, if any, in spermatogenesis is not understood, they will here merely be described as seen in the spermatocytes.

The status of the mitochondria and Golgi bodies, from this standpoint, is uncertain. There is evidence that they sometimes divide or fragment in a manner somewhat analogous to the division of chromosomes; but whether they do this regularly enough to be considered as permanently self-perpetuating is still a question, although in the case of the mitochondria some writers (cf. Gatenby,) consider the evidence convincing (see also p. 1285). In any event both the mitochondria and Golgi bodies take a prominent part in spermatogenesis.

The same is true of the centrioles, which are not only prominent in the germ cells, but are known to play a rôle in fertilization, and are probably transmitted from generation to generation like the chromosomes. The only question as to their genetic continuity is that raised by the fact that in artificially treated eggs cytasters may be seen to arise de novo. But it is not certain that these asters contain centrioles actually homologous to those in the normal amphiasters.

There remain only the chromosomes. These bodies, being self-perpetuating and playing a major rôle in the transmission of inherited characters (as is now well known), are constituents of especial interest—a fact made manifest by the almost innumerable studies devoted to them.

As a result of the studies just mentioned, the general history of the chromosomes during gametogenesis is well known—more so, for example, than that of the mitochondria and Golgi material. It will be convenient, therefore, to treat the chromosomes first and then return to the history of the other components later.

1. *Chromosomes:*

To simplify the discussion a general outline of chromosome transformations, applicable to most animals, and apparently to all mammals, may be given at this point. In considering this, as well as later details, it is essential to recognize one basic fact; namely, the chromosomes are present in two sets or haploid groups, one from the mother and one from the father. Appar-

ently it is the interaction of homologous members of these two groups which underlies the ofttimes elaborate and complicated processes taking place in gametogenesis.

During the growth period of the primary spermatocyte the chromosomes usually become drawn out into long, delicate "leptotene" threads, which later unite side by side in pairs, the members of each pair being homologous maternal and paternal derivatives. This process of "synapsis," often of an intricate nature and frequently followed by other complicated movements of the chromosomes, serves to reduce by half the apparent number of chromosomes, each of which is then double or "bivalent." Both components of the bivalents subsequently divide, giving rise to a quadripartite or "tetrad" structure—not always visible as such, however.

When cell division takes place, the chromosomes are thus superficially only half as numerous as before, and are quadripartite in structure (tetrads). Each tetrad divides into two double or dyad chromosomes, which pass to opposite poles. As a result, each second spermatocyte receives the half or "haploid" number of double chromosomes. These undergo relatively little change, and at the succeeding division separate into their two components, which in turn pass to opposite poles. The resulting cells are the spermatids, each of which contains the haploid number of single chromosomes. Thus each spermatozoon, like each egg, transmits the haploid chromosome group, and fertilization restores the double or diploid number.

As indicated by this outline, the stages of particular interest here are those found in the first spermatocyte. Subsequent ones are of relatively little significance, save in certain special cases.

(*a*) CHROMOSOME BEHAVIOR IN DIPTERA

One of the simplest types of spermatogenesis, and one which will serve to illustrate the general scheme of chromosome behavior, is that found in certain Diptera (flies). In this group of insects, as distinguished from most other animals, the chromosomes of the spermatogonia (also of body cells) are not only *present* in pairs, representing maternal and paternal homologues, but are *associated* in pairs—the two homologues ordinarily remaining side by side during the mitotic cycle. As a result, these homologues enter the primary spermatocyte already associated. In other words, synapsis (using the term in its broader sense) has already taken place.

These features are illustrated in Figures 593 to 597 which show the chromosomes at successive stages in the spermatogonia of such a fly. Figure 593 shows the five pairs of chromosomes during prophase when the homologues are intimately associated. Figure 594 represents them in metaphase, when the association is least conspicuous, and Figures 595 to 597 show them in successive stages of anaphase and telophase as the association again becomes intimate. They enter the primary spermatocyte, therefore, in the form of five double or bivalent chromosomes (Fig. 597).

These chromosomes now become diffuse (Fig. 598) for a short time, during which they lose their sharp outlines and can only be distinguished as cloudy aggregates. But almost immediately their staining capacity reappears, along with a process of condensation or

re-aggregation, and they again stand out as five distinct bodies. These are more elongate, thread-like and irregular in contour than before, and one is composed of two distinctly different portions—a thread, like the others, and a large, dense mass, usually more or less spherical (Figs. 599 and 600). The latter is the nucleolus. This body is probably compound, and calls for special note here.

At first it is uniformly dense, but later it becomes differentiated into two portions, one less deeply staining than the other (Fig. 601). Part, at least, of the deeply staining portion, together with the attached thread, is to be considered as representing the sex chromosomes (a feature shown clearly by other species of Diptera, as well as other insects). This portion, then, is not to be considered as truly nucleolar in the sense used in the classification given above. The remainder of the nucleolus is presumably made up of "true" nucleolar or "plasmosome" material.

In this case, as in many others, it is difficult to make a clear distinction between the basophilic (chromatic) and oxyphilic (plasmosome) materials in the nucleolus. Indeed, so far as the writer is aware, no consistent differentiation based on staining reaction is possible. In some cases the same body will take the acid plasma stains at one stage and the basic chromatin stains at another stage in the same cell.

Returning to a consideration of the chromosomes, we find them undergoing little visible change, except in size, as the growth period progresses, until the stage shown in Figures 602 and 603 is reached. Then they become more or less ribbon-like, with their duality plainly visible, and they become polarized with respect to the nucleolus, to which they are all attached. This polarization persists for some time and is accompanied by a tendency toward contraction or clumping together of the threads.

After this period is passed the chromosomes break loose from the nucleolus —except the small one which has been attached to it from the beginning —and then all undergo a rapid process of condensation into short, thick chromosomes preparatory to division (Fig. 604).

The two maturation divisions follow in rapid succession, giving rise to four sperms, each with five chromosomes. Examples of such division figures are seen in Figures 605 and 606.

(*b*) OUTLINE OF GROWTH STAGES

In such a case as the above, the changes undergone by the chromosomes appear to be relatively simple, and are easily followed. It should be emphasized, however, that such conditions are exceptional, and also that they are probably secondarily acquired, not primitive. As already mentioned, most organisms differ from these in that the chromosomes are not associated in pairs preceding the maturation stages. Also in the other organisms, the maneuvers of the chromosomes during the growth stages are almost always of a more complicated sort. We cannot here go into details respecting these, but we may outline some of the stages passed through by the chromosomes in more "typical" cases.

For instance, Wilson (1925*a*, p. 537) lists a series of eleven different stages found in many animals. These are enumerated below, together with a brief characterization or discussion of each.

1. *Final Gonial Telophase.* Here the chromosomes are single (univalent) and are beginning to diffuse (Fig. 607).

PLATE II

FIGS. 593–606.—Chromosome behavior during spermatogenesis in the robber fly, Asilus sericeus, Say. (After Metz and Nonidez.)

FIG. 593.—Spermatogonial prophase showing the 10 chromosomes intimately associated in pairs.

FIG. 594.—Metaphase, after dissociation of members of pairs.

FIG. 595.—Spermatogonial anaphase showing paired association of chromosomes as they go to the poles.

FIG. 596.—Late anaphase or early telophase of final spermatogonial division showing intimate paired association of chromosomes.

FIG. 597.—Same—the pairing so intimate that almost all trace of duality has gone.

FIG. 598.—Four cells in late telophase after the chromosomes have begun to lose their staining capacity.

FIG. 599.—Early growth stage of primary spermatocyte after the staining capacity is regained.

FIG. 600.—Somewhat later—five bivalent chromosomes present, one attached to nucleolus.

FIG. 601.—Later growth stage showing elongation of bivalents and differentiation of nucleolus into two regions.

FIGS. 602 and 603.—Contraction and post-contraction stages, showing the appearance of duality in the ribbon-like bivalents and their attachment to the nucleolus.

FIG. 604.—Prophase showing condensation of bivalents.

FIGS. 605 and 606.—First and second spermatocyte divisions, respectively.

PLATE II

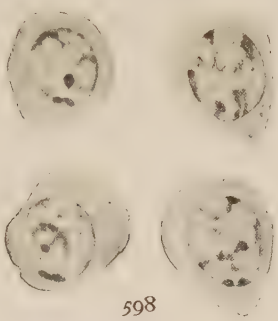
593

594

595

596

597



598

599

600

601

602

603

604

605

606

2. *Resting Stage.* In this stage the chromosomes become spun out, or diffuse, and make up a network in which the individual members cannot be distinguished.

3. *Prochromosome Stage.* Here the chromosomes become more condensed and can be distinguished as separate aggregates. They are still single (Fig. 608).

4. *Unravelling Stage.* Such a stage is transitional and marks the spinning out or uncoiling of the chromosomes to form long, delicate threads (Fig. 609).

5. *Leptotene Stage.* After the threads have become spun out they remain for a time in this "leptotene" form (Figs. 610 and 611).

6. *Synapsis Stage.* This is the most significant of the stages, and represents the culmination of the earlier or preliminary stages through which the chromosomes have gone preparatory to uniting in pairs. The union takes place, in many cases at least, by means of a side-by-side approximation of the delicate leptotene threads. This often begins at one end and passes progressively throughout the length of the threads (Fig. 612). In such cases the threads are usually polarized toward one point (the idiozome—see below) and form the so-called "bouquet." Or they may contract into a dense knot, defying detailed analysis.

7. *Pachytene Stage.* This stage follows the completion of synapsis, and in it the chromosomes are seen as heavy threads, fewer in number than before (Fig. 613)—in suitable cases clearly half the preceding number. Their duality is usually not clear until the succeeding stage.

8. *Diplotene Stage.* As the name implies, the double structure of the bivalent chromosomes becomes visible here, and sometimes the two paired threads separate rather widely from one another (Fig. 614).

9. *Diffuse or Confused Stage.* This stage, the significance of which is unknown, but presumably physiological, is somewhat analogous to a resting stage. The chromosomes become diffuse and are often difficult to trace.

10. *Second Contraction Stage.* Here, also for an unknown reason, the chromosomes again become contracted or clumped together.

11. *Diakinesis or Propbase.* During this period, which is often divisible into three separate stages, the chromosomes (in the form of tetrads) may go through complex changes in form, and may present various bizarre figures (cf. "General Cytology," Sect. x). Such figures are presumed to result from earlier intimate relations of the two synaptic mates, and their daughter halves, which together make up the tetrad. These phenomena, as a whole, are probably concerned with an interaction, and in some cases with an interchange of materials, between the four components. When such interchange is manifest genetically, it is called "crossing over" (cf. "General Cytology," Sect. xi). It should be observed, however, that cytologically it is extremely difficult to demonstrate such interchange or "crossing

over," and, furthermore, that in the males of some species at least, such crossing over does not take place. For instance, in the several species of Diptera (*Drosophila*) thus far studied, crossing over is found only in females. This should introduce caution into any interpretation of such figures where genetic studies have not shown that crossing over really takes place. This is particularly true in insects. In mammals crossing over has been shown to occur in both sexes, and it is a fair presumption that the condition is widespread in this group.

It is evident from this outline that in most forms the individual chromosomes cannot be traced through the various growth stages. This fact, together with similar inability to trace them through the resting stages of other cell generations, long left it uncertain as to whether or not the individual chromosomes actually retained their identity during these stages. The evidence for the continuity and "individuality" of the chromosomes is now conclusive, however, and need not be dwelt on here. It is reviewed in most modern textbooks on cytology or genetics.

We come to look upon the chromosomes, then, as prime examples of living, self-propagating constituents or organs of the cell. That they are complex in structure and that each possesses a definite architecture is made practically certain by modern studies on genetics, supplemented by direct cytological observation. As mentioned above, the evidence is equally conclusive that they are in large part responsible for the transmission of inherited characters. In these respects they stand alone among the cellular components.

Many modifications of the above processes are found. In some cases there is no synapsis of chromosomes, in others the chromosomes appear to unite only at their ends (telosynapsis as contrasted with parasynapsis) and in still others some chromosomes may undergo an intimate synapsis, while others merely touch (apparently). The first condition is found especially in species hybrids, where it is assumed that the failure of synapsis is due to dissimilarity between the chromosomes from the two respective parental species. Particularly significant are the cases in which the parents are from closely related species and some of the chromosomes undergo synapsis, while others do not. The differences in behavior are believed to represent differences in degree of likeness of the respective "homologues" or corresponding members.

These, and many other lines of evidence, lead to the now generally accepted view that the underlying cause of synapsis is the likeness of the respective maternal and paternal homologues. Such a statement gives no adequate explanation of the phenomenon, however. There is more than that involved; but just what the additional factors are is at present uncertain. One of the most puzzling situations, for instance, is that presented by certain hermaphroditic worms in which synapsis occurs during spermato-

PLATE III

FIGS. 607–636.—Final spermatogonial telophase in the orthopteran Phrynotettix. (After Wenrich.)

FIG. 607.—Prochromosome stage in the hemipteron Lygaeus, showing the univalent chromosomes in the form of diffuse masses, except the sex chromosomes which are condensed. (After Wilson.)

FIG. 608.—Unravelling stage in the hemipteron Largus, showing the transformation of the prochromosome masses into threads. (After Wilson.)

FIG. 609.—Leptotene stage in Phrynotettix. (Cf. Fig. 606.)

FIG. 610.—Leptotene stage in the mouse. (After Federley.)

FIGS. 611 and 612.—Synaptic stage and early parchytene in Phrynotettix. (Cf. Fig. 606.)

FIG. 613.—Diplotene stage in the mouse. (Cf. Fig. 610.)

FIGS. 614, 615 and 616.—Chromosomes of man. Figs. 614 and 615. Spermatogonial groups from a white man, showing 48 chromosomes. One of the sex chromosomes (Y) is identified by its small size. The other (X) is indistinguishable from many of the "autosomes." Fig. 616. First spermatocyte anaphase from a negro, showing segregation of X and Y. (After Painter.)

FIGS. 617 and 618.—Chromosomes of man. Fig. 617. A, spermatogonial metaphase plate—47 chromosomes. Fig. 618. Chromosomes of same figure arranged in pairs according to size—the unpaired X at left. (After Winiwarter and Oguma.)

FIGS. 619–627.—Chromosomes and maturation stages in marsupials. Fig. 619. Spermatogonial chromosome group of Pseudochirus perigrinus, showing 20 chromosomes, including the unequal sex chromosomes X and Y. (After Altmann and Ellery.)

FIGS. 621–624.—Phascolarctus cinereus. Fig. 621. Chromosome group of female (16 chromosomes) including the two similar sex chromosomes (XX). Figs. 622 and 623. Corresponding groups from the male, showing dissimilar sex chromosomes (XY). Fig. 624. First spermatocyte division showing separation of X and Y. Figs. 625–627. Ptauroides volans (Agar). Fig. 625. Late pachytene stage of first spermatocyte. Fig. 626. Early diplotene stage; XY, condensed sex chromosomes; Px, plasmosome. Fig. 627. Early second spermatocyte of same. (After Greenwood.)

FIGS. 628–631.—Chromosomes of various mammals. Fig. 628. Spermatogonial group from ring-tailed monkey (54 chromosomes). Figs. 629 and 630. Portions of first spermatocyte figures in same, showing sex chromosomes (XY). Fig. 631. Segregation of sex chromosomes at first spermatocyte division in opossum (A), monkey (B), and man (C); X above, Y below. (After Painter.)

FIG. 632.—Primary spermatocyte of guinea pig, showing nucleus, mitochondria, Golgi apparatus, acrosome, centrioles, axial filament, etc. (The proximal centriole and proximal half of distal centriole are both applied to the nuclear wall here.)

FIG. 633.—Scattered idiozome substance and Golgi rodlets of Hemiptera. In this form they apparently do not collect to form a definite idiozome mass at any stage.

FIGS. 634–636.—Mitochondria in living primary spermatocytes of the orthopteran Chortippus curtipennis. Fig. 634. Late prophase showing two masses of granular mitochondria (m). Ch, chromosomes. Fig. 635. Unstained. Metaphase showing mitochondrial threads migrating toward the poles of the spindle. Fig. 636. Telophase showing the breaking up of the filaments and their separation into two groups. Ch, chromosomes; m, mitochondria. (After Lewis and Robertson.)

PLATE III

607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
X
Y
m
ch.

genesis but not during oogenesis *in the same individual* (cf., e.g., E. Krüger, 1913; P. Hertwig, 1920).

2. *Chromosomes of man and other mammals:*

In man and other mammals chromosome studies have been impeded by technical difficulties and, as a rule, by the large number of chromosomes present. In man, e.g., it is not yet certain as to the exact number of spermatogonial chromosomes. According to Painter (1923), the number is 48 (Figs. 615 to 617), but according to de Winiwarter and Oguma (1926), it is only 47 (Figs. 618 and 619). The difference is due to an uncertainty regarding the sex chromosomes (cf. "General Cytology," Section X for discussion of sex chromosomes). The latter authors believe that the conspicuous X-chromosome has no mate, so that only one sex chromosome is present. Painter, however, describes and figures a small Y-chromosome behaving as a mate to the X, and undergoing segregation from it (Fig. 617). Since each of these conditions is widespread in other animals, it makes little theoretical difference as to which is ultimately found to exist here.

Especially favorable mammalian material has recently been found by Agar and his students in the Australian marsupials (cf. Agar, 1923; Greenwood, 1923; Altmann and Ellery, 1925). The accompanying figures (Figs. 620 to 627), taken from their papers, show typical chromosome groups and a few stages in spermatogenesis. In all of these species the males possess an unequal pair of sex chromosomes (XY). Likewise in the monkeys (Figs. 629 to 631) and the opossum (Fig. 631) and probably in the horse, bat and rabbit, dimorphic sex chromosomes (X and Y) are present (Painter, 1925).

So far as known, the maturation processes in mammals are in all essentials entirely comparable with those in other animals.

3. *Cytoplasmic constituents:*

Turning to the cytoplasmic components of the germ cells, we have to consider especially the centrioles, mitochondria and Golgi bodies, together with the "idiozome material." Among the recent reviews dealing with these elements may be mentioned those of Bowen (1922*a*, *b*, 1924), Gatenby (1917 to 1920), Wilson (1925*b*), and Duesberg (1920), from which the present account is largely derived.

Briefly stated, the general history of the above components is, in many cases at least, as follows: The centrioles, aside from functioning as astral centers in the spermatocyte divisions, are conspicuous as the central components of the "idiozome" in the primary spermatocyte (Fig. 637A), and also as definite organs in the sperm, one functioning as a basal body from which the axial filament of the sperm tail grows out (Fig. 637B). The mitochondria are present in the cytoplasm of the spermatocytes in the form of granules, rods, filaments or larger bodies (Fig. 637A, Figs. 634, 635, 650, 651) and are transmitted to the sperm, where at first they often form a definite body, the "nebenkern." The Golgi bodies, like the mitochondria, usually appear first in the form of small granules or rods, which during the growth stages typically collect about the idiozome (Fig. 637A). Later they pass into the spermatid, where they again typically aggregate about

the idiozome material to form the "acroblast" (Fig. 637B). Part of the latter then becomes the "acrosome," while the remainder is usually cast off.

Such an outline is, of course, a very general statement. As in the case of the nuclear components, many different conditions exist in different organisms, and we cannot hope to go into the details of these here.

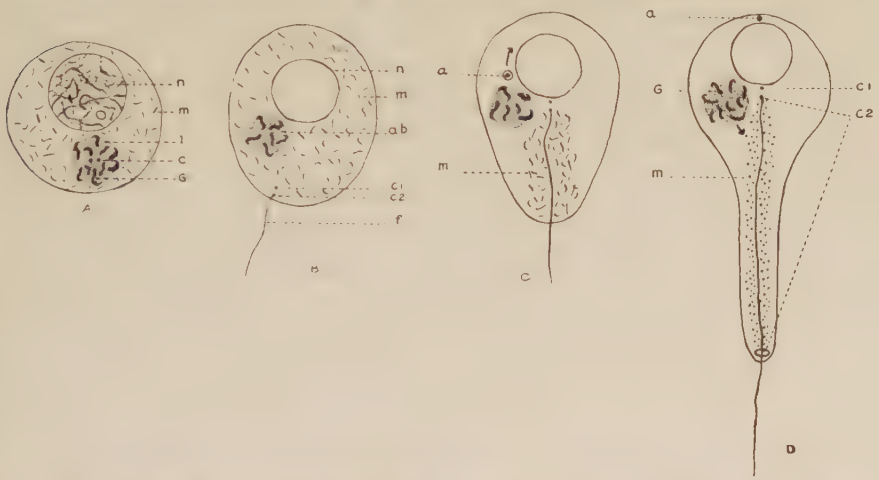


FIG. 637.—Diagrams representing the cytoplasmic constituents at various stages in spermatogenesis. A. Growth period of primary spermatocyte. B. Young spermatid. C and D. Later stages in transformation of spermatid: a, acrosome; ab, acroblast; c, centrosomes; c1, c2, proximal and distal centrosomes respectively; f, flagellum; G, Golgi bodies; i, idiozome; m, mitochondria; n, nucleus. (After Bowen.)

(a) CENTROSOMES, GOLGI BODIES AND IDIOZOME MATERIAL

Attention may first be directed to the centrosomes, Golgi bodies and "idiozome material," since these are correlated in their activities.

During the early growth period of the primary spermatocyte the idiozome as a whole, or the "Golgi body idiozome complex," is frequently seen in the form of a spherical mass, eccentrically placed in the cell (Fig. 632 and Fig. 637A). In the center are the two centrioles. About these is a region of fairly uniform density, composed of the "idiozome material" which ordinarily does not stain deeply. At the periphery of the latter lie the Golgi bodies, commonly in the form of deeply staining rodlets, fairly evenly scattered about the surface. Often these appear to form a network, or even a more or less continuous envelope, but such conditions are believed to be due, probably, to faulty technique. (A special, and difficult, technique is required for any detailed study of these bodies.)

When the centrioles separate and move to opposite poles—the time varying from early to late in the growth period, according to the organism—the idiozome usually breaks up and the Golgi bodies scatter. Sometimes,

however, as in the case of the scorpion *Opisthacanthus* (Wilson, 1925*b*), the idiozome may divide bodily into two parts which move along the nuclear membrane to the spindle poles (Figs. 641 and 642). In the latter case the Golgi bodies and idiozome material obviously remain associated. In other cases it is believed that each Golgi body retains a small amount of idiozome substance attached to it (Bowen, 1922*b*). These compound bodies then either segregate into two groups, which eventually pass to the poles, or else they fragment and the fragments undergo segregation.

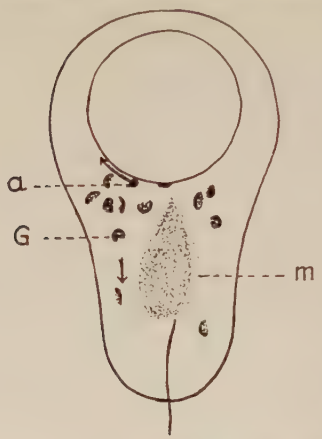


FIG. 638.—Diagram illustrating the formation of the acrosome from the multiple type of acroblast in certain Orthoptera. Symbols as in Figure 637. (After Bowen.)

In the second spermatocyte a somewhat similar process takes place, so that eventually each spermatid receives a number of Golgi bodies with (presumably) idiozome substance attached.

The apparent connection or association between Golgi material and the idiozome substance has led to the view (cf. Bowen, loc. cit.) that these two components are organically connected and should be considered as representing one structure—the rodlet and idiozome substance together constituting a Golgi body.

This view definitely separates the idiozome from any connection with the archoplasm or centrosome substance found at the astral centers during mitosis. It also harmonizes with the fact that in many forms (e.g., Hemiptera) the idiozome substance and Golgi rodlets are apparently scattered at all stages, and do not collect to form a definite idiozome mass (Fig. 633).

(*b*) MITOCHONDRIA

The history of the mitochondria runs parallel in many respects to that of the Golgi bodies. In the early spermatocyte growth period they are usually small and numerous, as in the spermatogonia (Fig. 632). Later they may remain scattered, or they may aggregate to form one or more granular masses (Fig. 634) definitely localized in position; or they may fuse in groups to form large chondriosome spheres or rods (Fig. 640); or in the extreme case of the scorpion *Centrurus*, considered below, they may coalesce to form a single body (Fig. 646). During mitosis they are often grouped about the spindle, frequently forming strands parallel to the spindle fibers (Figs. 635 and 636). Whatever their form, they ordinarily segregate bodily at the spermatocyte divisions, without themselves undergoing division. In at least one case, however, an accurate fission occurs as a regular process. This is

the case of the scorpion *Centrurus*, which has been fully investigated by Wilson (1916, 1925*b*). Since in other genera of scorpions a very different situation is found, we may use members of this group to illustrate certain types of mitochondrial behavior. At the same time they will serve to bring out additional characteristics of Golgi body and centrosome behavior.

In the scorpion *Opisthacanthus* (Wilson, 1925*b*) the mitochondria are present in spermatogonial and early first spermatocyte stages as numerous very small rodlets or granules. During the early growth stages these fuse in groups to make relatively large spheres approximately constant in number (±24). By about the middle of the growth period, the first spermatocyte appears as shown in Figure 640. Here the chondriosome spheres (c) are scattered about more or less at random. The idiozome (i) is also prominent here, applied to the nuclear wall at one side. During the remainder of the growth period, and also during the first division, the chondriosomes remain essentially unchanged except for an increase in size, due to active growth. Figures 641 to 643 show them at successive prophase stages, scattered about the periphery of the nucleus or about the mitotic figure as it makes its appearance. In Figures 642 and 643 the elongate bodies (ch) are chromosomes, and the spherical ones chondriosomes. During these stages the idiozome, including the Golgi material, often divides into two portions (Fig. 641) which move apart along the nuclear wall, and eventually follow the centrosomes to the poles (i, Fig. 642 and g, Fig. 643). In other cases the idiozome may break into smaller fragments which, however, appear to have the same later history. In metaphase and anaphase (Fig. 644) the Golgi material lies at the poles about the centrioles. When division occurs the chondriosomes separate into two groups of approximately twelve each and pass to the poles (Fig. 644).

In the second spermatocyte they remain relatively unchanged and at division again separate into two groups so that each spermatid ordinarily receives six. Thus an equal distribution of the original twenty-four (approximately) is effected, but without division of any of the chondriosomes themselves.

In another scorpion, *Centrurus exilicauda* (Wilson, 1916), as mentioned above, the opposite type of behavior is found; i.e., the chondriosomes divide by an accurate process of fission. In the present case the fusion of the mitochondrial filaments continues until all are united in one body—a thick ring. In the anaphase of the first division this ring extends lengthwise of the spindle between the two polar groups of chromosomes (Fig. 645). Then it constricts at the poles and the halves straighten out to form two rods (Fig. 646) which undergo constriction at the middle (equator of the spindle) and send daughter halves to the two poles. At the second division the two daughter rods divide by a similar process (Fig. 647) and in turn send daughter halves to either pole.

Thus in *Centrurus*, as in *Opisthacanthus*, an accurate and equal distribution of chondriosome material is effected, but by a widely different process. It would seem at first that the actual chondriosome materials in these cases must be essentially different,

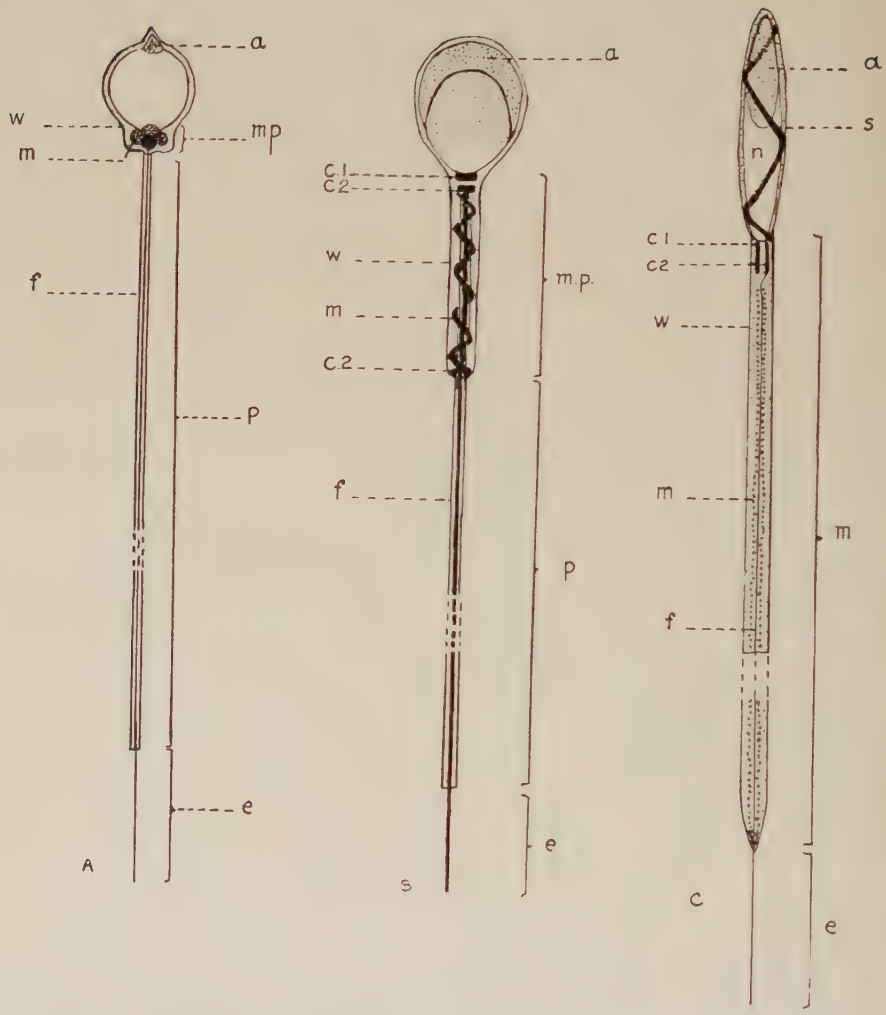


FIG. 639.—Diagrams of typical sperms. A, Based on echinoderm, annelid and mollusc. B, Based on mammal. C, Based largely on insect and mollusc: s, skeletal fiber in head; w, cell membrane; mp, middle-piece; p, principal-piece; e, end-piece; other symbols as in Figure 637. (After Bowen.)

but their previous and subsequent history gives no support to such an assumption, for the chondriosome bodies are similar in origin and in fate, giving rise in both cases to the nebenkern and then to the tail envelopes of the sperm.

Another interesting comparison of chondriosome behavior is that made by Gatenby (1919) in the case of the mollusc *Paludina vivipara*. Here, as has long been known, two kinds of sperms—the "typical" and the "atypical"—are formed by the same animal. The difference between these has been traced back by Gatenby to the spermatogonia, and possibly to the primordial germ cells. In the "typical" series the mitachondria are relatively large rods, few in number—a condition rare in animals. In the "atypical" series, on the other hand, they are small and granular and very numerous—a condition more common in other animals.

This distinction is well shown by the primary spermatocytes at the end of the growth period, as indicated by Figures 650 and 651. An equally characteristic distinction is seen in the mode of distribution of the mitochondrial material at the spermatocyte divisions. In the typical series the large chondriosome rods frequently (but apparently not always) undergo fission (Fig. 652) much as in the case of *Centrurus* cited above. But the granular mitochondria of the atypical series appear to be separated bodily into two groups without themselves undergoing division or taking any active part in the process (Fig. 653).

These few examples, taken more or less at random, serve to show the lack of general uniformity in mitochondrial behavior. Unlike the chromosomes the mitochondria cannot as yet be brought with certainty into one broad scheme which will give a conception of their structure, behavior and function. It may be emphasized, however, that this goal may not lie far off. A sufficient advance has been made in the last few years to lead Wilson, in a recent review of the subject, to generalize by saying that the chondriosomes are "probably . . . variable, plastic and polymorphic forms assumed by a specific substance which is *per se* capable of extended and perhaps unlimited growth; which arises from a preexisting substance of the same kind, and is genetically continuous in successive generations of cells; but which is not definitely or permanently organized to form individualized structural units" (*1925b*, p. 490).

It is also not unlikely, as Wilson points out in the same connection, that this statement may be found to hold for the Golgi bodies.

4. *Sperm formation:*

(*a*) DEVELOPMENT OF THE SPERMATID

As already observed the four spermatids receive from the primary spermatocyte approximately equal complements of the various cellular components, namely, chromosomes, mitochondria, Golgi bodies, centrosomes and general cytoplasm. In addition, there are often present chromatoid bodies and other stainable materials, but the distribution of these is usually irregular and their significance unknown; hence they will be left out of account here.

(*b*) GENERAL SKETCH

In the newly formed spermatid, the nucleus appears essentially as in other cells, as do also the centrioles. The mitochondria and Golgi bodies are usually (but with marked exceptions) scattered about in the form of small granules or filaments. Very soon after this the Golgi bodies usually collect into a group and an anterior-posterior differentiation of the spermatid becomes visible. In the mammalian spermatid, which may be used for illustration, conditions at this stage are roughly as shown in Figure 63–B. The Golgi bodies and idiozome material have collected at one side of the nucleus to form the acroblast (ab) which looks essentially like the idiozome of the first spermatocyte, minus the centrioles. The latter are situated near the periphery of the cell in alignment with the nucleus. From the peripheral one (c2) a flagellum (f) grows out which even at this early stage may be seen in active movement (Duesberg). This later becomes the tail filament. From its first appearance it marks the posterior end of the spermatid.

As development proceeds the acroblast becomes differentiated into two parts, due to the formation within the original body of a small vesicle containing a granule. The vesicle and granule together are called the acrosome (a). After its appearance the acrosome gradually separates from the parent body (Fig. 63–C) and coincidently its position becomes more and more anterior, so that eventually it comes to lie at the anterior end of the nucleus opposite the centrioles (Fig. 63–D). Meanwhile the bulk of the acroblast, or the Golgi remnant (G) passes in the opposite direction and comes to lie free in the cytoplasm—being eventually cast off.

The forward shift of the acrosome is probably caused by the rotation of the nucleus and by its passage to the tip of the spermatid. During these stages, most of the mitochondria (m) become shifted toward the tail region and aggregated about the base of the flagellum and the centrosomes—the latter having meanwhile moved in toward the nucleus.

The proximal centriole becomes applied to the nuclear wall, where it is frequently invisible; and the distal one divides into two portions, a granule and a ring (Fig. 63–D). The granule remains stationary, but the ring passes back for some distance along the flagellum, which it encircles. Much of the flagellum now becomes enclosed in a thin sheath, apparently its own product.

Later stages mark the elongation of the tail, the aggregation of the mitochondria about the axial filament and the casting off of the surplus cytoplasm, including the Golgi remains and in many cases other visible bodies such as chromatoid bodies and surplus mitochondria.

The sperm then comes to look approximately as shown in Figure 639B. The acrosome has expanded into a cap over the anterior end of the nucleus, and these two structures together definitely make up the head of the sperm.

Behind the head is a long narrow region, the "middle piece" (m.p.), including the centrioles, mitochondria and such cytoplasm as remains in the sperm. The mitochondria have united to form a spiral thread (m) which winds about the axial filament between the two components of the distal centriole —i.e., most of the length of the middle piece. Behind the middle piece are two regions, the long "principal piece" (p) consisting of the filament and surrounding sheath, and the "end piece" (e) consisting of the naked filament.

The details of sperm formation vary considerably in different mammals, and as will be noted later, they exhibit almost endless diversities in other animals; but the underlying features seem to be essentially alike throughout.

(c) SPERM FORMATION IN THE OPOSSUM

As an example of spermiogenesis in mammals we may use Duesberg's (1920) work on the opossum, together with that of von Korff (1902) on this and other marsupials. Conditions here are not entirely typical, but Duesberg's account has the advantage of including observations on all the main cellular components studied with the aid of modern developments in technique.

Figures 654 to 656 show young spermatids fixed according to different methods to reveal the mitochondria (654 and 655) and the acroblast or Golgi-body-idiozome material (656). The latter has already differentiated into the acrosome sphere and the residual portion (stained black in Figure 656). These figures also show the position of the nucleus, centrioles, flagellum, etc.

In Figure 657 is seen a later stage after the nucleus has taken an anterior position and has rotated so that the acrosome lies at the apex opposite the centrosomes. The latter have moved in toward the nucleus and the residual portion of the acrosome is seen near them. The mitochondria have undergone little change in position but are becoming spherical instead of filamentous.

Following this stage the acrosome becomes so closely applied to the nucleus as to be almost invisible and the nucleus elongates transversely across the head of the spermatid (Fig. 658). At the same time the distal centriole gives off a relatively large granule, which later increases in size and eventually disappears or is cast off with the residual cytoplasm. Behind the nucleus is a broad collar region which is not found generally in mammals and apparently has no special significance. Later the nucleus becomes V-shaped (Fig. 659) and the mitochondria (mostly) collect around the base of the axial filament (Fig. 660) after the distal centrosome has divided and the ring part moved back along the filament. Apparently the proximal centriole divides also, for two additional granules are commonly present here. But the process has not been studied sufficiently to make sure of this point.

When the various components have become thus definitely located, the residual cytoplasm, including Golgi remnants, some mitochondria and other granules, is thrown off by way of the head (Figs. 661 and 662). In many other animals it passes off along the tail or from the side of the sperm. After the surplus cytoplasm is cast off, the mitochondria elongate and fuse together forming a closely coiled spiral around the axial filament (Figs. 663 and 664), in an apparently typical manner. Other changes at the same time, however, are atypical and need only brief mention. The V-shaped nucleus rotates so that its apex comes to point forward (Fig. 664) making the head somewhat spear-shaped. The structure is bilaterally asymmetrical and after the sperms are mature they commonly unite in pairs in such a way that the two members of a pair are mirror images of each other (Fig. 665).

PLATE IV

FIGS. 640–644.—Spermatocytes of the scorpion Opisthacanthus. (After Wilson.)

FIG. 640.—Middle growth period; idiozome (I) surrounded by Golgi bodies; chondriospheres (c) scattered about in cytoplasm.

FIG. 641.—Early first division prophase; nucleus in contraction stage (chromosome threads contracted into a sphere at one side of nucleus).

FIG. 642.—Idiozome (I) dividing; centrioles visible at the poles; chromosomes (Ch) and chondriospheres (c) scattered about.

FIG. 643.—Late prophase; Golgi masses (G) passing to poles; chromosomes and chondriospheres much as before.

FIG. 644.—First spermatocyte anaphase; Golgi masses at poles; chromosomes almost to poles; chondriospheres separating into two groups.

FIGS. 645–649.—Sperm formation in the scorpion Centrurus exilicauda. (After Wilson.)

FIG. 645.—First spermatocyte anaphase; chromosomes clumped at poles, chondriosome ring extending between these clumps ready to divide.

FIG. 646.—Telophase of same, showing division of ring into four parts.

FIG. 647.—Second spermatocyte telophase; chromosomes again at poles; indicates second (final) division of chondriosome rods by median constriction.

FIG. 648.—Young spermatid showing large nucleus and double nebenkern made up of the two chondriosomes.

FIG. 649.—Sperm showing early stage in elongation and twisting of the double nebenkern to form the spiral envelope of the flagellum.

FIGS. 650–652.—Spermatocytes of the mollusc Paludina. Centrosome (C), chondrioplast or Golgi (nebenkern) rod (CP), rods at equator (CMR), granular mitochondria (GM), mitochondrial rods (MR), nucleus (N), Golgi apparatus idiozome complex (AR and GP). (After Gatenby.)

FIG. 650.—Full-grown "typical" spermatocyte.

FIG. 651.—Nearly full grown "atypical" spermatocyte of same, showing nucleus, etc., and granular mitochondria.

FIG. 652.—Telophase of "typical" spermatocyte at second division, showing constriction of the four mitochondrial rods.

FIG. 653.—Somewhat earlier stage in corresponding division of "atypical" form showing irregular distribution of chromosomes and mitochondrial granules. Golgi material at CP.

FIGS. 654–665.—Sperm formation in the opossum. (After Duesberg.)

FIGS. 654–656.—Young spermatids fixed in different ways to show scattered mitochondria, acroblast with Golgi material at its periphery, centrosomes, flagellum, etc.

FIGS. 657–664.—Progressively later stages in transformation of the spermatid (see text for explanation).

FIG. 665.—Paired mature sperms.

PLATE IV

C
I
640
I
N
641
I
Ch
C
642
G
Ch
C
G
643
Ch
G
C
644
645
646
647
648
649
M.R.
CP.
AR
M.R.
650
AR+CP
GM
651
N
CMR
MR
652
CP
C
GM
CP
GM
653
654
655
656
657
658
659
660
661
662
663
664
665

From the above account it is evident that the nucleus (chromosomes) and centrioles are transmitted bodily by the sperm. The same is true of most of the mitochondria, although some of them are apparently discarded. The Golgi-body-idiozome material is for the most part cast off, but a definite part of it remains in the acrosome, so that essentially its transmission may be comparable to that of the mitochondria—a view supported by the fact that in some animals each one of the original Golgi bodies apparently contributes to the formation of the acrosome. The latter structure, on account of its position and appearance, has been considered as a perforatorium, but recent observations suggest that instead of being a mechanical instrument it may be a conveyor of materials for activating the egg (cf. Hyman, 1923; Bowen, 1923).

These features probably apply generally to animal sperms, even though the widest variations exist in the details of sperm formation. We can here note only a few examples of such variations.

In some cases the Golgi bodies do not fuse to form an acroblast but are scattered about in the spermatid (Fig. 638). In other cases the acrosome, instead of forming a point or cap at the tip of the sperm, may appear in a lateral position or even (certain Hemiptera) be drawn out along the side of the nucleus for almost the entire length of the head.

The mitochondrial structures also exhibit great diversity. Commonly they aggregate into a more or less dense body—the nebenkern—which later undergoes various changes, giving rise to a variety of structures. The nebenkern itself is frequently (e.g., many insects) onion-like in structure, made up of overlapping layers or plates. By some observers it was believed to arise from the "spindle remains" in the spermatid, but its origin from mitochondria now appears to be well established. In some invertebrates the mitochondria clump to form one or more dense bodies which lie at the base of the nucleus in the mature sperm (Fig. 639A). In others they may be scattered about in the middle piece, or they may appear in the form of single or double ribbons winding about the axial filament—as in the case of the scorpion *Centrurus* considered above (Fig. 649).

The centrosomes exhibit even wider modifications in structure, although, like the mitochondria, they regularly lie in the middle piece (in typical flagellate sperms). Some examples of centrosome modifications are noted below under sperm structure. In other cases these bodies (or usually the distal one) form loops, rings, threads, etc., of many types. But here again an underlying similarity in origin and development is common to all.

IV. MORPHOLOGY OF SPERMATOZOA

Spermatozoa as a whole may be grouped into two general classes: the *flagellate* and the *non-flagellate*. The former usually consist definitely of a head region and a tail region, the tail including a single flagellum. This type

is common to most animals and to all vertebrates. The non-flagellate, as the name implies, have no flagellum. These are found in relatively few animals and are almost certainly derived from flagellate sperms, hence they need not be considered here.

1. *Flagellate sperms:*

The flagellate sperms are again broadly divisible into two classes, the typical and the atypical; and again conditions in one group (the atypical) represent only secondary modifications of those found in the other.

Typical flagellate sperms are divided by Bowen (1924) into three groups, differing in the form and location of the mitochondria, cytoplasm and centrioles. These are represented diagrammatically in Figure 639. Bowen distinguishes primarily between the latter two types, using the first mainly to show the derivation of the other two; but for present purposes we may recognize all three as distinct.

In the first type (A), found in several groups of invertebrates, the sperm is divisible into four regions; (1) *the head*, consisting of the nucleus (n) and the small acrosome (a); (2) the short *middle piece* (mp) in which lie the condensed mitochondrial bodies (d) and the cytoplasm; (3) the "*principal piece*" (p) of the tail, which consists of the axial filament (f) and a surrounding sheath, without any cytoplasm, and (4) the *end piece* (e) consisting solely of the naked filament. In this type, then, the cytoplasm and the mitochondria are clumped in a short region and the middle piece is practically part of the head.

The second type (B), characteristic of mammals, differs from the first mainly in the greater prominence and different structure of the middle piece, although the acrosome may be considerably larger. The middle piece includes the same components as before, but they are here drawn out into a long structure surrounding the axial filament for a considerable portion of its length. The centrioles, which in type A are concealed by the surrounding mitochondria, are here distinct and prominent, one (c.1) lying at the base of the nucleus, and the other divided into two parts, a granule and a ring, as described above. Between these two parts and around the filament (with its enclosing sheath) is the spiral mitochondrial thread, also described above.

By a further elongation of the middle piece and a rearrangement of its parts the third type (C) is obtained. This type is found mainly in insects. Its differs from the others more or less in all its parts, but the two primary distinctions involve the centrioles and the mitochondria together with the cytoplasm. The centrioles lie side by side at the base of the middle piece instead of being disposed along its length, and the middle piece itself, with its enclosed cytoplasm and mitochondria, extends far out along the axial filament, completely eliminating the "principal piece" as a topographical region.

On this basis of classification, then, the middle piece is delimited by the extent of the cytoplasm and mitochondria, rather than by the position of the centrioles, as considered by some authors. For examples of the almost endless variety of animal sperms now known, the reader is referred to the figures of Retzius (1902 to 1912).

V. PATHOLOGICAL AND EXPERIMENTAL MODIFICATION OF THE GERM CELLS

Only a few aspects of this broad topic can here be considered, and discussion of these will be limited to conditions in mammals. In most mammals the mature testes instead of being retained in the abdomen are located at or near the periphery of the body in the scrotum. In rare individual cases this descent to the scrotum fails to take place. Such undescended ("cryptorchid," "ectopic" or "high flanker") gonads have long been known to be imperfectly developed or degenerate, with consequent sterility of the individual if both gonads are affected.

In these cases, the condition is not associated with any known pathological agent, but appears to be due solely to the abnormal position of the gonads. In other cases, however, a similar modification is found to result from pathological conditions (e.g., various diseases) or artificial treatment. For instance, it has long been known that in (scrotal) mammals, testes transplanted from one individual to another do not develop and mature as do ovaries, save under certain special conditions as indicated below. Likewise, artificial treatment with roentgen rays, radium, sera and other agents has been found to inhibit the development of the germ cells or to cause their degeneration. And, finally, it has been found that normal, mature testes if returned from the scrotum to the abdomen soon undergo degeneration and become like those which have never descended or have been artificially injured.

It is not to be assumed that conditions are exactly alike in testes modified in these different ways, but there is apparently an underlying similarity throughout, as far as the germ cells are concerned, and it is even probable that a common agent is responsible in many of the cases.

This latter feature is stressed by Moore in a series of papers, one of which (1926) includes a comprehensive review of the whole subject with abundant references to the literature. The present résumé is based largely on these papers.

1. *Cryptorchidism, vasectomy, vasotomy, transplantation, etc.* (*see also* pp. 1230, 1232);

In cases of cryptorchidism one or both testes may fail entirely to descend from the original abdominal position, or may descend only part way. If the testis does not reach the scrotum the germ cells fail to mature. If it protrudes part way into the scrotum, however, a portion of it may sometimes show normal conditions of spermatogenesis. When one testis descends normally and the other fails to descend the one exhibits normal spermatogenesis and the other does not.

These facts indicate, as already mentioned, that spermatogenesis here depends on the position of the testis, and that it can only take place normally if the testis is descended into the bottom of the scrotum (Griffiths, 1894). Various other lines of evidence confirm this view. For instance, if an immature testis is artificially returned to its abdominal position spermatogenesis does not take place, and if a mature testis is similarly returned, germ cell degeneration occurs (Griffiths, 1893). Likewise in certain hibernating mammals in which the testes only descend into the scrotum at certain seasons, spermatogenesis proceeds normally only during these periods.

Such evidence led Moore (1922) and Fukui (1923), working independently on the problem, to suggest that the immediate causal agent here is temperature—a suggestion made also by Crew (1922) on theoretical grounds.

Subsequently, by a series of experiments on various animals, Moore and his collaborators have supported this view in a convincing manner. The evidence follows two lines, one tending to show that other possible agents are not the effective ones, and the other showing directly the effect of temperature changes. Most convincing perhaps is the fact that direct application of heat to the scrotum causes degeneration apparently exactly like that caused by a return of the testis to the abdomen. Also it has been shown that the scrotal and abdominal temperatures are actually different. In guinea pigs and rabbits, e.g., the difference ranges from 1½° to 3°C. at a room temperature of 30°C.; and in the rat it may reach 7°C. at a very low room temperature (16°C.).

On this basis, then, as Moore points out, it is possible to view a series of phenomena in a common light, and perhaps to explain numerous conflicting accounts in the earlier literature. In such a category come not only the cases considered above, but also those including vasectomy, vasotomy, disease and transplantation.

Vasectomy and Vasotomy. It has been claimed by some writers that in cases of vasectomy or vasotomy a degeneration of the germ cells occurs comparable to that noted above. According to Moore, however, if the operation is properly performed and the position of the testis is not altered, degeneration does not occur. But if the testis is moved slightly into a region of higher temperature, as is apt to be the case, a cryptorchid condition results, with consequent effects on the germ cells due to the temperature and not to occlusion of the vas.

Degeneration Accompanying Disease, Etc. Similarly, clinical observations have indicated that in men the germ cells are affected by various diseases including, e.g., fevers, peritonitis, pneumonia, and other pulmonary infections all of which tend to increase the temperature of the patient. Such evidence, together with that indicating the absence of such degeneration in cases of death from other causes, tends to indicate that temperature is the responsible agent here.

With such cases, perhaps, may be classed those in which degeneration follows the injection of tuberculin into the testis after previous sensitization of the animal (Long and Seyfarth, 1924; Seyfarth, 1924).

Transplantation. Again in the case of transplantation experiments, temperature appears to play the deciding rôle. The evidence for this has largely been indicated above in the cases involving artificial cryptorchidism. It remains merely to note that successful transplantation followed by spermatogenesis is only obtained when the testis is placed in its normal position at the bottom of the scrotum.

2. *Degeneration following dietary deficiencies, or treatment with alcohol, roentgen rays, etc.:*

In a different category from the preceding are the cases in which degeneration is caused by the direct action of injurious agents or by deleterious conditions, unaccompanied by temperature changes. Among such cases are to be mentioned those involving, (1) treatment with roentgen rays, radium or (probably) alcohol, and (2) dietary deficiencies. Although the causal agents are different here, the visible effects on the germ cells are the same as in the preceding cases (f.c Moore, 1926, also Mason, 1925; Evans, 1925; Parkes and Drummond, 1925).

3. *Changes involved in degeneration:*

The morphological changes undergone by the germ cells in the various cases considered above are of a comparatively fixed and definite type. Whatever the means employed to influence them, the cells appear to be affected in sequence, and the sequence is the reverse of that shown by the stages of spermatogenesis. When the mature testis is involved, e.g., the first cells to degenerate are the spermatozoa; then follow the spermatids, the spermatocytes, etc. Similarly, if the embryonic testis is involved, or the agent is continuously active, as in natural cryptorchidism, it is the late stages that are absent or abnormal.

Where cryptorchidism is involved, the conditions have long been known, especially in man. Here the ectopic testes appear to be more or less normal until the time at which spermatogenesis should take place; then as the spermatocytes start to develop, degeneration sets in. Apparently the Sertoli cells and early spermatogonia are affected comparatively little; but the spermatocytes are, for the most part, unable to develop, and the few that reach the spermatid stage go to pieces without forming spermatozoa. Indeed, in most recorded cases no spermatids are found, and in many the spermatocytes and definitive spermatogonia are also absent. Here the seminiferous tubules are usually well rounded but are smaller and are filled with a syncytium of Sertoli cells.

In experimental animals also, the process of degeneration may go so far as to destroy the spermatogonia and apparently leave only the Sertoli cells (Griffiths, 1893). An approximate idea of the speed of the reaction is obtained from Moore's (1924) experiments on guinea pigs, in which marked degeneration of the germinal epithelium took place within six days after the testis was returned to the abdomen, and almost complete degeneration was reached by the twentieth day -leaving only Sertoli cells and a few spermatogonia. The length of time necessary to eliminate all the germ cells, if that is possible by this means, has not been determined. Moore found that a testis left in the abdomen twenty-four days and then returned to the scrotum had regained its normal structure and possessed mature sperm three months later.

When degeneration is caused by roentgen rays, or similar agents, or by direct application of heat, it is again the spermatozoa which are first affected. This is indicated both by cytological observations and by the duration of the period of sterility which follows such treatment. In cases of this kind the period of sterility is roughly proportional to the length of treatment.

The evidence taken as a whole leaves little question that in general the final stages in germ cell formation are most easily disturbed and that cells in earlier stages are progressively less sensitive in a more or less definite sequence back to the early spermatogonia—a conclusion applying not only to mammals but apparently to other groups of animals as well.

VI. BIBLIOGRAPHY

Agar, W. E. 1920. *Cytology —with special reference to the Metazoan nucleus.* London. MacMillan Co.

——— 1923. The male meiotic phase in two genera of marsupials (Macropus and Ptauroides). *Quart. J. Micr. Sci.*, **67**, 183.

Allen, B. M. 1904. The embryonic development of the ovary and testis of mammals. *Am. J. Anat.*, **3**, 89.

——— 1906. Origin of the sex cells in Chrysemys. *Anat. Anz.*, **29**, 218.

——— 1907. An important period in the history of sex cells of Rana pipiens. *Ibid.*, **31**, 339.

Allen, Edgar. 1923. Ovogenesis during sexual maturity. *Am. J. Anat.*, **31**, 439.

Altmann, S. C. A., and Ellery, M. E. W. 1925. The chromosomes of four species of marsupials. *Quart. J. Micr. Sci.*, **69**, 463.

Arai, Hayato. 1920. On the postnatal development of the ovary (albino rat) with especial reference to the number of ova. *Am. J. Anat.*, **27**, 405.

Beccari, N. 1924. Studi sulla prima origine delle cellule genitali nei vertebrati. III. Ricerche nel Bufo viridis. *Arch. Ital. di Anat. e di Embriol.*, **21**, 332.

Boveri, Th. 1887. Ueber Differenzierung der Zellkerne während der Furchung des Eies von Ascaris. *Anat. Anz.* (Jena).

——— 1892. Die Entstehung des Gegenstatzes zwischen den Geschlechtszellen und den somatischen Zellen bei Ascaris megalocephala. *Sitz. Ges. Morph. Phys. München*, 8.

Bowen, R. H. 1922*a*. Studies on insect spermatogenesis. II. The components of the spermatid and their role in the formation of the sperm in Hemiptera. *J. Morph.*, **37**, 79.

— — 1922*b*. On the idiozome, Golgi apparatus, and acrosome in the male germ cells. *Anat. Rec.*, **24**, 159.

——— 1923. The origin of secretory granules. *Proc. Nat. Acad. Sc.*, **9**, 349.

——— 1924. Studies on insect spermatogenesis. VI. Notes on the formation of the sperm in Coleoptera and Aptera, with a general discussion of flagellate sperms. *J. Morph.*, **39**, 351.

Crew, F. A. E. 1922. A suggestion as to the cause of the aspermatic condition of the imperfectly descended testis. *J. Anat.*, **56**, 98.

Doncaster, L. 1920. *An introduction to the study of cytology*. Cambridge Univ. Press.

Duesberg, J. 1920. Cytoplasmic structures in the seminal epithelium of the opossum. *Carnegie Inst. Wash., Pub. 272*, 49.

Evans, H. M. 1925. Invariable occurrence of male sterility with dietaries lacking fat soluble vitamin F. *Proc. Nat. Acad. Sci.*, **11**, 373.

Federley, H. 1919. Beiträge zur Kenntnis der Säugetiergametogenese. I. Die Spermatogenese von Mus silvaticus L. *Acta Soc. Sci. Fenn.*, **48**, 5.

Firket, J. 1920. On the origin of germ cells in higher vertebrates. *Anat. Rec.*, **18**, 309.

Foley, J. O. 1926. The spermatogenesis of Umbra limi with special reference to the behavior of the spermatogonial chromosomes and the first maturation division. *Biol. Bull.*, **50**, 117.

Fukui, N. 1923. On a hitherto unknown action of heat ray on testicles. *Japan Med. World*, **3**, 27.

Gatenby, J. B. 1917–20. The cytoplasmic inclusion of the germ cells. *Quart. J. Micr. Sci.*, **62**, **63**, **64**.

— — — 1924. The transition of peritoneal epithelial cells into germ cells in Gallus bankiva. *Ibid.*, **68**, 1.

Geiser, S. W. 1924. Sex ratios and spermatogenesis in the Top Minnow. *Biol. Bull.*, **47**, 175.

Greenwood, A. W. 1923. Marsupial spermatogenesis. *Quart. J. Micr. Sci.*, **67**, 203.

Griffiths, J. 1893. The structural changes in the testicle of the dog when it is replaced within the abdominal cavity. *J. Anat. and Physiol.*, **27**, 482.

— — 1894. Retained testes in man and in the dog. *Ibid.*, **28**, 209.

Hargitt, G. T. 1924. Germ cell origin in the adult salamander. *J. Morph.*, **39**, 63.

——— 1925. The formation of the sex glands and germ cells of mammals. I. The origin of the germ cells in the albino rat. *Ibid.*, **40**, 517.

— — 1926. The formation of the sex glands and germ cells of mammals. II. The history of the male germ cells in the albino rat. *Ibid.*, **42**, 253.

Hegner, R. W. 1914*a*. Studies on germ cells. I. The history of the germ cells in insects with special reference to the Keimbahn determinants. II. The origin and significance of the Keimbahn determinants in animals. *J. Morph.*, **25**, 375.

——— 1914*b*. *The germ cell cycle in animals*. MacMillan Co.

Hertwig, P. 1920. Abweichende Form der Parthenogenese bei einer Mutation von Rhabditis pellio. *Arch. f. mikr. Anat.*, **94**, 303.

Jordan, H. E. 1917. Embryonic history of the germ cells of the Loggerhead turtle. *Carnegie Inst. Wash., Pub. 251*.

Kahle, W. 1908. Die Paedogenese der Cecidomyiden. *Zoologica* (Stuttgart), **21**.

King, H. D. 1908. The oögenesis of Bufo lentiginosus. *J. Morph.*, **19**, 369.

v. Korff, K. 1902. Zur Histogenese der Spermien von Phalangista. *Arch. f. mikr Anat.*

Krüger, Eva. 1913. Fortpflanzung und Keimzellbildung von Rhabditis aberrans. *Zeit. wiss. Zool.*, **105**, 87.

Kuschakewitsch, S. 1910. *Die Entwicklungsgeschichte der Keimdrusen von Rana esculenta.* Fest. f. R. Hertwig. Jena.

Long, E. R., and Seyfarth, M. 1924. The testicle as an indicator of allergy in the hypersensitiveness of infection and anaphylaxis. *Am. J. Tuberculosis*, **9**, 254.

Mangold, O. 1925. Die Bedeutung der Keimblätter in der Entwicklung. *Die Naturwiss.*, **11**, 213, 231.

Mason, K. E. 1925. A histological study of sterility in the albino rat due to a dietary deficiency. *Proc. Nat. Acad. Sci.*, **11**, 377.

Moore, C. R. 1922. Cryptorchidism experimentally produced. *Anat. Rec.*, **24**, 383.

——— 1924. Testicular reactions in experimental cryptorchidism. *Am. J. Anat.*, **34**, 269.

——— 1926. The biology of the mammalian testis and scrotum. *Quart. Rev. of Biol.*, **1**, 1.

Okkelberg, P. 1921. The early history of the germ cells in the Brook Lamprey, Entosphenus Wilderi (Gage), up to and including the period of sex differentiation. *J. Morph.*, **35**, 1.

Painter, T. S. 1923. Studies in mammalian spermatogenesis. *J. Exper. Zool.*, **37**, 3.

——— 1925. A comparative study of the chromosomes of mammals. *Am. Nat.*, **59**, 385.

Parkes, K. S., and Drummond, J. C. 1925. The effect of vitamin B deficiency on reproduction. *Proc. Roy. Soc.*, **96**(B), 147.

Retzius, G. 1902–12. *Biologische Untersuchungen* X–XVIII. Stockholm, Jena.

Robinson, A. 1918. The formation, rupture, and closure of ovarian follicles in ferrets and ferret-polecat hybrids and some associated phenomena. *Roy. Soc. Edinburgh*, **52**.

Rubaschkin, W. 1912. Zur Lehre von der Keimbahn bei Säugetieren. *Anat. Hefte*, **46**, 343.

Seyfarth, McH. 1924. The abortion reaction in the testicle as an indicator of the hypersensitiveness of infection. *J. Infect. Dis.*, **35**, 489.

Sharp, L. W. 1926. *Introduction to cytology.* Ed. 2. New York: McGraw-Hill.

Simkins, C. L. 1923. On the origin and migration of the so-called primordial germ cells in the mouse and the rat. *Act. Zool.*, **4**, 241.

Stern, Curt. 1926. Vererbung im Y-Chromosom von Drosophila melanogaster. *Biol. Zentrlb.*, **46**, 344.

Swift, C. H. 1915. Origin of the definitive sex cells in the female chick and their relation to the primordial germ cells. *Am. J. Anat.*, **18**, 441.

Swingle, W. W. 1921. The germ cells of Anurans. I. The male sexual cycle of Rana catesbeiana Larvae. *J. Exper. Zool.*, **32**, 235.

—— 1926. The germ cells of Anurans. II. An embryological study of sex differentiation in Rana catesbeiana. *J. Morph.*, **41**, 441.

Waldeyer, W. 1870. *Eierstock und Ei.* Leipzig.

Wenrich, D. H. 1916. The spermatogenesis of Phrynotettix magnus with special reference to synapsis and the individuality of the chromosomes. *Bull. Mus. Comp. Zool. Harvard*, **60**, 57.

Wilson, E. B. 1912. Studies on chromosomes. VIII. Observations on the maturation phenomena in certain Hemiptera and other forms, with considerations on synapsis and reduction. *J. Exp. Zool.*, **13**, 345.

——— 1916. The distribution of the chondriosomes to the spermatozoa in scorpions. *Proc. Nat. Acad. Sci.*, **2**, 321.

Wilson, E. B. 1925*a*. *The cell in development and heredity*. Ed. 3. New York: MacMillan.

——— 1925*b*. Protoplasmic systems and genetic continuity. *Am. Nat.*, **59**, 481.

Winiwarter, H. de, and Oguma, K. 1926. Nouvelles recherches sur la spermatogenèse humaine. *Arch. d. Biol.*, **36**, 99.

Witschi, E. 1914. Experimentelle Untersuchungen über die Entwicklungsgeschichte der Keimdrüsen von Rana temporaria. *Arch. f. mikr. Anat.*, Abt. **2**, **85**, 9.

Woodger, J. H. 1925. Observations on the origin of the germ cells in the fowl (Gallus domesticus) studied by means of their Golgi bodies. *Quart. J. Micr. Sci.*, **69**, 445.

Section XXXVII

THE SEMINAL VESICLES, PROSTATE AND BULBO-URETHRAL GLANDS

CONTENTS

Section XXXVII

PAGE

I. Seminal Vesicles 1303
1. Epithelium 1304
2. Cytoplasm 1305
3. Secretion granules 1305
4. Other cytological constituents 1307
II. Prostate Gland 1308
1. Epithelium 1309
2. Secretion granules 1310
3. Lipoid 1311
4. Mitochondria 1312
5. Pigment 1312
6. Basal cells 1312
7. Basement membrane 1312
8. Concrements 1312
9. Duct epithelium 1313
10. Nerves 1313
III. Bulbo-urethral Glands 1313
1. Special features 1314
2. Epithelium 1314
3. Cytoplasm 1315
4. Tubular outlets 1315
IV. Bibliography 1316

Section XXXVII

THE SEMINAL VESICLES, PROSTATE AND BULBO-URETHRAL GLANDS*

CHARLES CLIFFORD MACKLIN

THE nidamental tissues of the male human subject are represented mainly by the secreting epithelium of three organs, the seminal vesicles, the prostate and the bulbo-urethral glands, whose products mingle with the spermatozoa in the upper end of the urethra. This epithelium forms a complete lining for the acini and storage compartments of these bodies, and passes without interruption into their ducts. That of the seminal vesicles develops from the mesoderm of the Wolffian duct, while in the prostate and bulbo-urethral glands the mother tissue is the entoderm of the urethra. The cells are highly differentiated as producers of one or other of the accessory reproductive secretions and, although varying materially in the different organs, yet they all possess the properties common to glandular tissues.

I. THE SEMINAL VESICLES

The epithelium of the seminal vesicles overlies the conspicuous elevations and deep depressions which give to sections of these twin sacs the simulation of tubular glands and villi. Its area is quite wide, for the mucosa, when detached from its fibromuscular capsule, extends as an irregular tube, abounding in blunt diverticula, for some ten or twelve centimeters (McMurrich, 1914), although in the undissected vesicle it is shortened to less than half this length by reason of marked coiling and folding.

The name "seminal vesicles" is not appropriate, since they are really glands of external secretion, and act as reservoirs for only one element of the seminal fluid, which they themselves elaborate, and discharge at comparatively rare intervals. Among those who deny that they are sperm reservoirs and group the organs with the accessory glands of reproduction are Kayser (1889), Limon (1901) and Oberndorfer (1902). A few, as Rehfisch (1896), Lode (1896) and others, while acknowledging their secretory function, yet hold that they normally contain spermatozoa in the human subject, and it is admitted that these elements may be found, though usually in small numbers, also in the seminal vesicles of other animals, as the guinea pig. Their presence, however, is probably to be looked upon as accidental.

Not all of those who agree that their function is secreting concede that this is related to reproduction. Thus, since Akutsu (1903*b*) found, in sexually immature rats, evidence of secretory activity, he felt that the product

*Figures in this section will be found on plates following bibliography.

in these cases at least could not subserve reproductive functions. Petersen (1907), too, observed that the gland begins to function at a very early age, for, even in the vesicles of a human fetus, he saw conditions which could, he thought, only be interpreted as of a secretory character, and in those of a nine-months-old boy he observed a mass in the lumen which seemed identical with that of the adult.

Such observations have cast doubt upon the connection of this secretion with sexual activities. Steinach's (1894) report that removal of the seminal vesicles of rats in no way affected sexual desire is in line with this view, but is not conclusive, since the prostate was also extirpated, and the later discovery that animals so operated upon were unable to fertilize females of proved fertility is in favor of the opposite opinion. The evidence of de Bonis (1909) that the height of secretory activity coincides with coitus, and that the sacs undergo marked diminution in productivity after castration, and that of Fisher (1923) that they atrophy after such a procedure points to a definite relation between the seminal vesicles and the sex function.

The secretion is weakly alkaline, clear and gelatinous, and is made up of small rounded masses like grains of cooked sago. It is not characteristically mucoid (Akutsu, 1903*b*, rat). The function is in doubt, and seems to vary in different animals. Szymonowicz (1924) suggests that it clears the urethra of sperm. In some animals, as the guinea pig, it is held to form a plug in the vagina to prevent the escape of sperm.

1. *Epithelium:*

The epithelial cells are said to rest directly upon the connective tissue or upon the walls of the capillaries, without the intervention of a basal membrane (Oppel, 1904*b*). There seems to be no general agreement as to the number of layers, some authorities, as Fränkel (1901), Akutsu (1903*b*, rat) and Oppel (1904*b*) affirming that there is but one; others, as Zimmermann (1898) and Kolster (1902), claiming that there are two, while still others state that there may be either one or two layers (Langerhans, 18–4). Hermes (1893) never found two layers, although he described some of the cells as growing so large as to overlap their neighbors. Kolster (1902) feels that the basal cells act as a repair depot for the surface cells which are lost during the period of sex activity in the animal.

Most authorities agree that the typical epithelial cell of the seminal vesicle is cylindroid in form, although a wide variety of shapes have been described, from low and flattened to high cylindrical. Guelliot (1883) depicts them as flat in the adult, Akutsu (1903*b*, rat) and Armitstead (1925, guinea pig) as cylindrical, and Langerhans (18–4) finds cylindrical cells if a second layer is present and flat cells if it is absent. Zimmermann (1898), in material from an executed criminal, reports cuboidal cells, between and under which are interspersed single basal cells only half as high, with

rounded upper borders (Fig. 2). Where these were numerous there were two complete layers, the under cells in this event being much flattened (Fig. 3). Eberth (1904*a*) feels that such diverse appearances are due to the varying functional states of the cells, or to the different ages of the subjects. We know, too, that the cells become shorter when the vesicle is distended.

The cell form is somewhat different in the recesses or crypts from that on the ridges, Fränkel (1901) finding it lower in the recesses, while Akutsu (1902) verifies this for the newborn, but reverses it for the adult. Some authorities, as Gegenbauer (1899), consider that the true glandular epithelium is to be found only in the depressions, while others, as Guelliot (1883) and von Duhot (1901), regard the entire epithelial lining as secretory. Eberth (1904*a*) states that the secretory activity is more abundant in the depths (Fig. 1) and on the heights of the folds than along their sides. Pohl (1923) finds that in cross section the surface cells have a polygonal outline.

The nucleus is large, of round or oval form, and has a distinct membrane and nucleolus. It lies near the center of the cell except when pushed basalward by secretion granules.

The cell outlines are distinct, and at the free end a condensation of granules may simulate, in some instances, a cuticular border. The cells of the new born possess a true cuticular border, according to Langerhans (1874), which persists until the age of sixteen. Zimmermann (1898) described a regular cement substance, with terminal bars, which holds together the upper row of cells (Figs. 2 and 3). Akutsu (1903*b*) finds that leucocytes occasionally make their way between the cells.

2. *Cytoplasm:*

The basal cytoplasm stains rather densely, and contains mitochondria, while in the peripheral region the secretion granules are the most conspicuous component (Fig. 11). Fat and pigment granules are also present. Zimmermann (1898) found that every cell showed two diplosomes, dissimilar in size and shape, the longer oval one lying nearer to the free end of the cell, and the more rounded one toward the basal end. Occasionally the upper cells possessed a flagellum (Figs. 2 and 3).

3. *Secretion granules:*

The secretion granules are the most characteristic cell constituents, and have been described in very early life, even in the human fetus, by Petersen (1907). This author finds, in any given human vesicle, all stages of the process of secretion, and Akutsu (1903*b*) discerned the entire gamut of functional change in all the seminal vesicles of the rat which he examined. It seems likely that the production of secretion is fairly constant, although its rate may be modified, particularly by copulation.

The staining reaction of the granules is described as partly basophilic (Limon, 1901) and partly acidophilic (Petersen, 1907). It is similar to that of the secretion in the lumen.

The picture of the cell varies enormously with the stage of activity. Akutsu (1903*b*) has given a complete account of the process of manufacture and discharge of secretion granules in the rat. Before the appearance of these, the cell (Fig. 4) is cylindrical in form, with a dense protoplasm in which numerous small and refractile granules may be found in the fresh condition. The rather lightly staining oval nucleus, containing one or more caryosomes, occupies a variable position, but is usually situated somewhat high in the cytoplasm. He describes numerous "Altmann's granules" (mitochondria) at the base of the cell, disposed in chains or rows about the nucleus. In the upper part of the cell, although numerous, they are not arranged in any definite order.

Secretion granules are first formed in the central core of the supranuclear zone (Fig. 5), and their appearance is accompanied by an increasing vacuolation of the cytoplasm in this region—indeed, it is described as a meshwork of protoplasmic strands containing the granules, which are of variable size, but are never as large as a red blood cell. They are sharply defined and strongly refractive, staining intensely with iron hematoxylin and other stains. They are dissolved by acetic acid. As the granules increase in number and size, the supranuclear region becomes swollen (Fig. 6) and the nucleus, which now stains more intensely, is pressed toward the base. It is even more markedly dislocated in Figure 7, representing a more advanced stage of granule accumulation, and its appearance suggests increase of chromatin. A distinct nucleolus is now visible. Here the granules have distended the cell so much as to give it a conical form, and they are beginning to be extruded into the lumen. The appearance of spaces around the granules is due to shrinkage. The cytoplasm is foam-like, with thin films separating the vacuoles, and the cell outlines are indefinite. The charged condition of the cell may not be characterized by such a typically conical form, Figure 8 showing cylindrical cells, in which the secretion granules have even invaded the basal region of the protoplasm.

The envelope of cytoplasm covering the superficial granules becomes thin and bursts, liberating them into the lumen, where they coalesce to form the gelatinous secretion. Deeper granules work upward to the surface, and are discharged, and this process leaves the cell protoplasm of an open, lace-like character, as shown in Figure 10, the cell borders appearing ragged, and the granule content being markedly diminished. Mitochondria become very sparse, and seem to be used up in the process—indeed, Akutsu surmises that they give rise to the secretion granules. After the granules have been discharged, the cell undergoes a period of reconstruction, in which it resumes the form seen in Figure 4, the homogeneous cytoplasm spreading

upward from the basal region, and the nucleus meanwhile losing its distinct nucleolus and becoming more lightly staining. It is then ready to begin again the cycle of granule elaboration. Akutsu is of the opinion that similar functional cell changes may occur in the vesicles of other animals.

The discharge of secretion is accomplished under the influence of the hypogastric nerve (Akutsu, 1903*a*, *b*), for when this is stimulated the cell changes from the loaded condition shown in Figure 9 to the discharged form seen in Figure 10.

Petersen (1907) gives a somewhat similar account for the process in the human subject, a man aged twenty years. In Figure 12 a quiescent stage is shown, the cytoplasm being filled with small acidophil granules. These decrease in amount (Fig. 13) as the weakly acidophil granules, of much larger size, form in the distal end of the cell, in the meshes of the reticular threads of this region. A still further stage is seen in Figure 14, where the secretion granules continue to increase at the expense of the protoplasmic granules, and the free border is beginning to be irregular, forming protoplasmic prolongations.

Stilling's (1884) findings in rabbits differ from the above principally in that the nucleus is described as more intensely stained in the resting than in the active cell. He found that the secretion granules, in this animal, were discharged during copulation.

The results of Armitstead's (1925) observations upon guinea pigs are, for the most part, in agreement with those of Akutsu. He describes very fine granules in cells exhausted by excessive copulation, which are massed at the free border and extend down the sides. The cytoplasm is more strongly acidophilic in the discharged than in the resting condition, and the cell borders are still definite.

A few workers, as Petersen (1907) and de Bonis (1909), hold to the nuclear origin of the material which becomes transformed into the secretion granules, but Akutsu (1903*b*) was unable to find any evidence of this, and most workers do not favor this view.

4. *Other cytological constituents:*

Cowdry (1918) described and figured (Fig. 11) the *mitochondria* of the seminal vesicle of the white mouse. Here they are typically long and thread-like, although short rods and intermediate forms also occur. They lie principally in the basal protoplasm, and extend up around the nucleus, in which region they lie with their long axes parallel with the secretory axis of the cell. A few may even occur among the secretion granules. They do not, according to Cowdry, show blebs, which are regarded as the precursors of secretion by some workers.

The epithelium contains a pigment which gives to the mucous membrane a characteristic brownish color (von Ebner, 1902; Akutsu, 1902). It

probably does not occur until puberty, since Maass (1889) never found it before the age of twenty, nor Petersen (1907) before sixteen. The golden-brown granules are described as polygonal, and as varying in size from fine particles up to the diameter of a red blood cell. The pigment increases with age until it fills the cell, occupying principally the supranuclear zone, and favoring the cylindrical rather than the basal cells. It is an iron-free lipochrome (Szymonowicz, 1924) and not doubly refractive (Kaiserling, 1910). The time of its appearance suggests its relation to the assumption of sex duties by the seminal vesicles, but the precise part, if any, which it plays, and its genesis, are obscure.

Fat occurs in the cells (Akutsu, 1903*b*, rat), and may form large drops, and in the basal cells may occupy almost the entire cytoplasm (Limon, 1901).

The epithelium is the site of inflammatory and degenerative disorders of various kinds (Adami and Nicholls, 1911) and may rarely be involved in carcinomatous change.

The epithelium of the ejaculatory ducts is characterized by the occurrence of four or five small diverticula of similar character to the seminal vesicles.

II. THE PROSTATE GLAND

The prostatic epithelium clothes the irregular walls of the secreting units of the organ, which are dispersed, in the human subject, through some thirty to fifty separate glands, wrapped up closely in a contractile fibromuscular stroma. In many lower animals, as the guinea pig, the arrangement is quite loose. The organ encircles the "prostatic urethra," which presents in cross section a triangular outline, with the apex pointing forward. The posterior wall projects into the lumen, forming the postero-lateral sulci, which receive the more important prostate ducts. These ducts are usually said to number from fifteen to thirty, although Lowsley (1912) finds them to average as high as sixty-three.

Young and Davis (1926) have recently synopsized the later work, notably that of Loeschke (1920) and Adrion (1922) on the minute anatomy of the prostate, from which it appears that, in cross section, three concentric regions of increasing size from within outward can be distinguished, which differ from one another in the character of the glands which they contain. The main mass is composed of the long and branched "external" or prostatic glands proper, leading into ducts which curve backward to enter the postero-lateral sulci. This thick outer layer invests a smaller mass of "inner" secreting elements, and is separated therefrom by a capsule, which Jacoby (1923) finds inconstant. The inner glands, in turn, present two groups: a larger outer set of submucosal units, fairly long and also branched, whose ducts empty beside those of the "external" set, and which surround the

relatively insignificant group of short and simple glands immediately enveloping the urethra. The latter are known variously as the mucosal, suburethral, central or periurethral glands, and have an important clinical significance.

The dimensions and weight of the prostate are important because of their susceptibility to variation, notably to pathological increase through hypertrophy of the epithelial elements. The dimensions, even for the normal adult, show a wide range, the diameters as given by Keyes (1923) being: length, 33 to 45 mm.; width, 34 to 51 mm.; thickness, 13 to 24 mm. Its weight is stated to be from 16 to 24 gms. The ratios of glandular to muscular tissue are as variable, being given by Cabot (1924) as 5:1; by Walker (1900) as 3:1; and by Deaver (1922) as about 1:1.

It has been associated with the accessory reproductive glands for a number of reasons, among others because of its marked development in the human subject at puberty, and in animals at the onset of the rutting season (Griffiths, 1890); and because of its failure to develop fully after castration, and its usual regression with old age.

The secretion, known often as the "succus prostaticus," is a thin, milky, weakly alkaline and odorous fluid containing a nucleo-protein, lecithin, choline and other materials. It is said to neutralize the acid urinary and vaginal tracts, and a function of activating the spermatozoa has been attributed to it, with which its high content of sodium chloride (1 per cent) has been associated (Cabot and Young, 1917); it is thought also to promote coagulation of the seminal vesicle fluid.

The secreting units are characteristic follicle-like dilatations which act as storage chambers, and contain masses of secretion and frequently one or more typical "prostatic concretions." Elevations of the irregular and folded walls often project into the lumen like villi (Fig. 15) and in cross sections may appear as islets of epithelial tissue. It is not to be wondered at that, due to the peculiar form of these units, there has been difficulty in classifying them. Maziarsky (1901), Weski (1903) and other workers regarded them as tubulo-alveolar, while not a few, as Hyrtl (1889) and Brösike (1897), look upon them as tubular, and Krause (1913) has supporters when he assigns them to the alveolar type.

1. *Epithelium:*

The epithelium differs somewhat in different regions of the same follicle, as well as in different follicles, and we find a striking lack of harmony in the descriptions of the numerous writers regarding almost every point of discussion.

Thus there is a notable disagreement as to the number of layers. Among those who describe a single layer of columnar cells are Walker (1910) for the adult rat, and Weski (1903) for the human subject. The latter author

occasionally finds a second layer of flattened elements crowded between the bases of the columnar cells, and here the structure is such that the nuclei of the latter are above the level of the free border of the former, thus giving the false impression of a double-layered condition. A number of workers consider two layers as normal, describing a stratum of flattened or cuboidal cells underlying the lining of cylindrical cells (Langerhans, 1874; Rüdinger, 1883; Disselhorst, 1904, for the human, as quoted by Petersen, 1909; Schafer, 1912; Prenant and Bouin, 1910, Fig. 15). Still others find beneath the cylindrical cells two layers of flattened cells (Athanasow, 1898). Walker (1899, 1900) states that the lumen of the human gland is obliterated by cells in the newborn, and that this type persists until about sixteen years of age.

It seems to be the favored opinion that the lining epithelium is more than one cell deep, and also that the surface cells are usually of the high cylindrical type (Fig. 15), although here cuboidal elements are frequently found. Some cells are narrow and darkly staining, while their neighbors are often wider and lighter (Prenant and Bouin, 1910). The stage of secretion and the degree of distention of the acinus have apparently much to do with the height of the cells.

The cell outlines (Fig. 15) are quite definite. The free border is often rounded or protruding, and occasionally retains its connection with the secretion in the lumen (Walker, 1900, 1910, rat and dog; and Weski, 1903, human). Underlying this border is a rather darkly staining, granular protoplasm. The basal zone may stain lightly or heavily, depending, apparently, on its functional condition. It is less granular, and composed of an open network of fine threads, in the meshes of which are a few fat granules. Walker (1900) was unable to demonstrate any trophospongium in the dog. There is considerable variation in shape, size and staining reaction. In transverse section the cells are pentagonal (Krause, 1913).

The nucleus is oval and vesicular, and possesses a clear outline. Its position varies in the cell, but is usually near the base.

2. *Secretion granules:*

The secretion granules are the most conspicuous bodies in the cytoplasm, and have been variously pictured. The figures of Petersen (1909) are here given, and were made from material taken from a twenty-four-year-old man. In Figure 16 we have a cuboidal, non-granular, coarsely reticular cell with a large, oval, centrally placed nucleus. Figure 17 represents a cell which Petersen regards as being in an early stage of secretion formation, with the cytoplasm occupied by rounded, basophilic granules of various sizes. These, in developing, change in their staining reaction to acidophilic, and we have in Figure 18 an intermediate stage in this transformation, for here many of the granules are partly eosinophilic and partly

basophilic. Gradually the entire granule is changed. Cells often contain granules of both types, as well as intermediate forms.

As development proceeds, the now elongated cell is crowded with small eosinophilic granules (Fig. 19) whose staining reaction is similar to that of the material in the lumen. The nucleus is sometimes in the center and sometimes pushed toward the base.

The mature acidophilic granules are discharged into the lumen (Fig. 20), and concomitantly the basal protoplasm clears, assuming a fibrillar or net-like structure. The peripheral region, however, remains at first densely staining, and is sometimes prolonged into the lumen (Fig. 20). Ultimately the entire cell returns to the condition seen in Figure 16.

Petersen found granules in the fresh condition which corresponded to those he described in the fixed cell.

Weski (1903) states that, after a period of secretion, the human cells may be quite flat, with a mere rim of protoplasm about the nucleus, and wide spaces between. As the secretion is re-formed, the cells enlarge, obliterate the spaces laterally, coming into contact with their neighbors, and show an accumulation of granules at the free border. This cycle is repeated again and again.

The basophilic granules were regarded by Weski (1903) as being the material from which the prostatic concretions were formed, but Petersen (1909), as we have seen, looks upon them as the early stage in the formation of the secretion granules, and as not related to the concretions in any way.

The prostate has been looked upon as a gland of intermittent secretion, but Petersen (1909) found evidence of continuous activity in the human subject. The rate of secretion is doubtless subject to considerable variation, Stilling (1884) having demonstrated that during copulation the cells change from the tall and granule-packed condition, in which the lightly staining nucleus is basally situated, into the low form, depleted of granules, with the centrally placed nucleus more definitely stained.

3. *Lipoid:*

Although certain writers, as Herxheimer (1904) and Munk (1908), have regarded the prostate as a fat-free organ, Petersen (1909) found granules in the basal region of the cells, which he interpreted as fat. Later, various workers described lipoids in the epithelial cells (H. Posner, 1911; C. Posner, 1912), some finding them present before puberty (Plenge, 1924), and others not until after puberty (Kaiserling, 1910; Hada, 1914). Ishihara (1915) found them most plentiful during the sixth and seventh decades, Björling (1903) considered them as mere débris from destruction of leucocytes in the gland, but Plenge (1924) finds too few leucocytes in the prostate normally to account for all the fat. In Figure 22 we have the lipoid represented as numerous small black granules collected mainly in the region of the nucleus,

with not a few mixed with the secretion in the lumen. This material was from the prostate of a forty-two-year-old man (Plenge, 1924).

Some of the fat was found to be doubly refractive (de Bonis, 1907, Kunze, 1922, for the dog; Sehrt, 1913, and Plenge, 1924, Fig. 23, for the human), and by some authors was considered to be part of the normal secretion of the organ (Rapoport, 1911; Fürbinger, 1911; Kawamura, 1911; Fischel und Kreibich, 1911; Randisi, 1912 and others). Kinoshita (1920), on the other hand, regarded it as due to changes consequent upon illness. Sehrt (1913) found the doubly refractive fat at all ages, but most pronounced during sexual life; Plenge (1924) only after puberty, and Schlagenhaufer (1909) in prostatic carcinoma.

The lipoid is more abundant in the epithelium of the ridges than in that of the furrows. It is in the form of fine droplets (Fig. 22) and seldom completely fills the cell, being more concentrated at the basal end. By appropriate staining technique the fat is found to be present in various forms, as neutral fat, fatty acids, cholesterin, lecithin and myelin.

4. *Mitochondria:*

The mitochondria are very minute and of granular form (Fig. 21), according to Cowdry (1918). Dominici (1913) found that the quantity varied with the activity of the cell, and regarded them as taking an indirect part in secretion formation, but as not becoming directly transformed into it.

5. *Pigment:*

A light yellow pigment of uncertain significance has been described in the prostatic epithelium by Petersen (1909).

6. *Basal cells:*

Petersen (1909) found that the flattened basal cells were more strongly acidophilic and more homogeneous in appearance than the cylindrical surface cells. In sections the nucleus often does not occur. Weski (1903) considers these flattened elements as corresponding to the tall columnar cells, but modified mainly through pressure and secretion changes.

7. *Basement membrane:*

A definite basement membrane is claimed by some (Oppel, 1904*a*; Eberth, 1904*c*) and denied by others (Weski, 1903; Schaffer, 1922).

8. *Concrements:*

The prostatic concretions, or "colloid masses," have been looked upon as inspissated secretion (Mansell-Moullin, 1895) and as products of degenerate prostatic cells apart from the real secretion (Eberth, 1904*c*; Oppel,

1904*a*). They are particularly abundant in old persons, and are spheroidal or egg-shaped bodies, ranging from about 0.3 mm. to 1.0 mm. or more in diameter. In section they present concentric layers, which have been deposited, apparently, around desquamated cells or cell fragments. They may become calcified, and certain staining reactions suggest amyloid at times.

9. *Duct epithelium:*

The duct epithelium is columnar (Disselhorst, 1897), except at the outlet, where it becomes transitional, like that of the bladder and prostatic urethra.

10. *Nerves:*

The prostate has both motor and secretory nerves (Mislawsky and Bormann, 1898). Timofeew (1895) has described a laminated corpuscular nerve ending which terminates in the muscle and glands of the organ. These structures are unique in that they have two myelinated fibers entering them, the thick one running through the core and ending in a knob, while the thin one breaks up into an elaborate plexus about the first. Their function is not understood.

A variety of infections, notably by the gonococcus, may attack the prostatic mucosa. Benign and malignant proliferations of the epithelial cells are common (MacCallum, 1924; Young and Davis, 1926). The importance of the small mucosal glands, in this connection, has already been stressed. The enlargement of a group of these on the posterior aspect of the urethra, first described by Jores (1894), was shown by Albarran and Motz (1902) to account for hypertrophy of the median prostatic lobe, which often leads to serious interference with micturition; indeed it is one of the most important single factors in the etiology of the obstructive uropathies (Young and Davis, 1926). They are frequently known as "Albarran's glands." Motz and Perearneau (1905) referred all prostatic hypertrophies to hyperplasia of the mucosal glands.

III. THE BULBO-URETHRAL GLANDS

The bulbo-urethral glands, or glands of Cowper, are paired, yellowish-brown bodies, each about the size of a pea, which lie one on either side of the membranous portion of the male urethra, embedded in a contractile stroma of striated muscle, intermixed with a few fibers of the smooth type, and connective tissue. They are of the branched tubulo-alveolar design, each opening by a slender duct, 3 to 4 cm. in length, into the floor of the bulbar portion of the urethra. Thin rings of smooth muscle surround the excretory ducts.

These small organs are usually considered to be accessory glands of reproduction. Besides the cytological changes in the alveoli, to be described, which attend copulation, we have additional evidence for this view in the observation that the glands enlarge at puberty (Eberth, 1904*b*; de Bonis,

1909) and atrophy in old age, and that of Schneidenmühl (1883) who found them less developed in castrated animals. On the other hand, Bilharz recorded that they are of the usual size in eunuchs (Eberth, 1904*b*); and Langerhans (1874) was unable to demonstrate any parallelism in development between them and the other sex glands.

The secretion is a clear, viscid, mucous substance, of neutral or weakly alkaline reaction, which gives no clouding with acetic acid (Henle, 1863; Stilling, 1885; Szymonowicz, 1924). It is said to be discharged into the urethra during sexual excitement, and is thought to lubricate the passageway for the aftercoming seminal elements, and possibly to assist in neutralizing the urethral walls.

1. *Special features:*

The gland is peculiar in a number of ways: (1) It possesses tubuloalveoli which are often dilated (Fig. 24) and vesicular, although this is not always the case, in some of them the lumen being narrow. They vary considerably in size and shape. These end pieces may terminate blindly, or (2) may communicate with one another (Braus, 1900) through intermediary tubules, thus producing a net-like structure. (3) The acini open directly, or through a narrow and short tubule (Fig. 25), into the ampulla of the duct. True intercalary ducts are said not to occur, and the presence of secretory ducts has been disputed. (4) The peripheral terminations of the excretory ducts, or ampullae, into which the aforementioned narrow tubules open, are remarkable in often being quite widely dilated (Figs. 24 and 25). They are lined by cuboidal epithelium, or even by flattened elements. The epithelium, however, varies in form here, narrow ampullae being found lined by cylindrical cells.

The aforementioned dilatations of the ducts and alveoli are for the purpose of storing the secretion which is discharged under the constricting influence of the enveloping muscle fibers. It seems more than possible that the gland actively produces and discharges secretion during the hyperemic condition attending sexual excitement, for it is obvious that its storage space would provide for only a small volume of fluid.

2. *Epithelium:*

The epithelium of the alveoli is usually of the simple columnar type, the cells having, as a rule, the form of cones, with the truncated and somewhat irregular apices projecting into the dilated lumen (de Bonis, 1909). Eberth (1904*b*) states that this border is usually well defined. Often the peripheral ends of the cells are separated from one another by extensions of the lumen, which form clefts between them, and these spaces have been termed intercellular secretory canaliculi, or capillaries (Braus, 1900, Fig. 26), although Schaffer (1922) is doubtful as to the appropriateness of such

a designation. In their gross arrangement they resemble mucous glands (Schaffer, 1922). They rest on a well-developed membrana propria.

The nuclei are large and oval, and are situated toward the base of the cell. They often appear crowded and compressed. There is a distinct nuclear membrane, a definite nucleolus, either centrally or peripherally placed, and a close chromatin network.

3. *Cytoplasm:*

The protoplasmic substrate is clear, and in this there are abundant secretion granules which, upon being dissolved out, leave a fine reticular structure (Fig. 26, after Braus, 1900). Schaffer (1922) describes these granules as oxyphil in reaction, and as being eliminated not only from the free end of the cell, but also from the adjacent sides, leading to appearances which, he says, have been interpreted as terminal bars.

There has been much discussion as to whether or not these cells are mucous in character. Braus (1900) finds the same variations in staining reaction in them that are found in the cells of the mucous glands of the mouth in the unstimulated condition. Eberth (1904*b*) and Schaffer (1922) also feel that they resemble mucous cells, the latter stating that they are filled with granules of mucigen. It is of interest that Müller (1892) found mucus in the cells of the homologous glands of a 16 cm. female fetus. Henle (1863), Stilling (1885) and Disselhorst (1897) obtain no positive indications that the cells are of the mucous type. It seems also to be doubtful whether or not there are any typical serous cells in Cowper's glands.

Schaffer (1922) has described small, spindle-shaped bodies in the protoplasm, mixed with the mucus, which he terms characteristic, and which stain with connective tissue stains, such as that of Mallory. Their significance is not stated. Sparse fuchsinophile corpuscles have also been described (de Bonis, 1909).

Stilling (1885) found evidence of discharge of secretion during copulation (Figs. 27 and 28). He pictures the resting cell as pyramidal, with a clear protoplasm, an oval nucleus near the base, and bordering on a small lumen. After copulation the tubules show, instead of a round cross section, an indented one; and the lumen is wide, irregularly bordered and filled with granular substance. Cell outlines have disappeared. The protoplasm of the gland cell has become dark and finely granular, and the nucleus is placed in the middle of the cell. Transition forms between the active and the resting cell are found, and are fewer the longer the gland remains inactive, having disappeared in animals that have not copulated for six weeks.

4. *Tubular outlets:*

There is more or less gradual transition in the type of epithelium from that of the alveoli, through the intermediate ductules, into the ampullary

dilatations of the ducts (Fig. 25), the cells becoming lower and more darkly staining and losing their secretion granules. Occasionally the duct cells are projected into the alveolus as in the case of the pancreas (Oppel, 1904c). Indications of the presence of mucus have been found in the duct cells. In the net-like ducts, connecting the alveoli, the epithelium is high, cylindrical and as darkly staining as in the larger ducts.

The main ducts are lined by a single layer of low cuboidal epithelium with indefinite borders, non-granular protoplasm and a round, centrally placed nucleus. Toward the mouth of the duct the elements are usually cylindrical, and are underlaid with flattened irregular cells, usually constituting two layers; there may even be a stratified cylindrical epithelial lining here (Schaffer, 1922). The glands are subject to infections, especially by the gonococcus, and to other disorders.

IV. BIBLIOGRAPHY

Adami and Nicholls. 1911. *Principles of pathology.* Ed. 2, Phila.: Lea and Febiger.

Adrion, Walter. 1922. Ein Beitrag zur Ätiologie der Prostatahypertrophie. *Ziegler's Beiträge zur pathologischen Anatomie und allgemeinen Pathologie,* **70,** 179.

Akutsu, Saburo. 1902. Beiträge zur Histologie der Samenblasen nebst Bemerkungen über Lipochrome. *Virchow's Archiv,* **168,** 467.

— 1903*a*. Beiträge zur Kenntnis der Innervation der Samenblase beim Meerschweinchen. *Archiv f. die ges. Physiol.,* **96,** 541.

— — 1903*b*. Mikroskopische Untersuchung der Sekretionsvorgänge in den Samenblasen. *Ibid.,* **96,** 555.

Albarran and Motz. 1902. Contribution à l'étude de l'anatomie macroscopique de la prostate hypertrophiée. *Ann. des maladies des organes génito-urinaires,* **20,** 769.

Armitstead, R. B. 1925. The structure, function and regeneration of the seminal vesicles of the guinea pig. *J. Exp. Zool.,* **41,** 215.

Athanasow, P. 1898. Recherches histologiques sur l'atrophie de la prostata consécutive à la castration, à la vasectomie et à l'injection sclérogéne. *J. de l'anat. et de la phys.,* **34,** 137.

Björling, E. 1903. Woraus bestehen die Prostatakörper? *Arch. f. Dermatol. u. Syphilis.*

de Bonis, V. 1907. Über die Sekretionserscheinungen in den Drüsenzellen der Prostata. *Arch. f. Anat. u. Entw.,* 1.

——— 1909. Sur les phénomènes de sécrétion dans les cellules glandulaires des vésicules séminales et des glandes de Cowper. *Arch. Ital. de Biol.,* **52,** 205.

Braus, H. 1900. Über den feineren Bau der Glandula bulbo-urethralis des Menschen. *Anat. Anz.,* **17,** 381.

Brösike, G. 1897. *Lehrbuch der normalen Anatomie.* Berlin: H. Kornfeld.

Cabot, H. 1924. *Modern urology.* In original contributions by American authors. Ed. 2., revised. Phila.: Lea and Febiger, Vol. 1.

Cabot, H., and Young, E. L., Jr. 1917. The prostate. Vol. vii. In *A Reference Handbook of the Medical Sciences,* Vol. vii. N. Y. Wm. Wood & Co., p. 323.

Cannieu, A. 1892. Recherches sur l'appareil reproducteur mâle du Cavia. *Revue des Sci. Nat. de l'Ouest,* **2,** Nos. 1 and 3, 55.

Cowdry, E. V. 1918. The mitochondrial constituents of protoplasm. *Contrib. to Embryol.,* No. 25, Vol, 8, the Carnegie Institution of Washington, p. 39.

Deaver, J. B. 1922. *Enlargement of the prostate.* Ed. 2, Phila.: Blakiston.

Disselhorst, R. 1897*a*. *Die accessorischen Geschlechtsdrüsen der Wirbeltiere mit besonderer Berücksichtigung des Menschen.* Wiesbaden.

——— 1897*b*. *Die accessorischen Geschlechtsdrüsen der Wirbeltiere. Eine vergleichend-anatomische Untersuchung.* Inaug. Diss., Tübingen, 36 pp.

——— 1904. *Ausführapparat und Anhangsdrüsen der männlichen Geschlechtsorgane.* Jena, 1904.

Dominici, M. 1913. Sulle formazioni mitocondriali e sui granuli di secrezione nella prostata del cane e prostata umana ipertrofica. *Folia urol.*, **7**, 295.

Dreyer, A. 1891. *Beiträge zur Pathologie der Samenblasen.* Dissertation, Göttingen.

von Duhot, R. 1901. Contribution à l'étude anatomopathologique des vésicules séminales. *Ann. d. mal. d. org. génito-urin.*, Paris, **19**, 769.

Eberth, K. 1904*a*. In *Handbuch der Anat. des Menschen*, edited by Bardeleben. Jena: G. Fischer. Seminal vesicles. In "Die männlichen Geschlechtsorgane," **7** (Abt. 2), 57.

——— 1904*b*. Cowper's glands. *Ibid.*, 164.

——— 1904*c*. Prostate. *Ibid.*, 140.

v. Ebner, V. 1902. In Kölliker's *Handbuch der Gewebelehre des Menschen.* Bd. 3, Leipzig: W. Englemann.

Eichbaum, F. 1879. *Über den Bau und die Funktion der Vesicula seminalis bei den Haussäugetieren. Vorträge für Tierärzte.*

Engle, E. T. 1926. The copulation plug and the accessory genital glands of mammals. *J. Mammalogy*, **7**, 119.

Felix, W. 1901. Zur Anatomie des Ductus ejaculatorius, der Ampulla ductus deferentis und der Vesicula seminalis des erwachsenen Mannes. *Anat. Hefte*, **17**, 1.

Fischel, R., and Kreibich, K. 1911. Über Prostatasekretion. *Wien. klin. Wochenschr.*, **24**, 901.

Fisher, N. F. 1923. The influence of the gonad hormones on the seminal vesicles. *Am. J. Physiol.*, **64**, 244.

Fränkel, M. 1901. *Die Samenblase des Menschen.* Berlin.

Friedmann, F. 1898. Beiträge zur Kenntnis der Anatomie und Physiologie der männlichen Geschlechtsorgane. *Arch. f. mikr. Anat.*, **52**, 856.

Fürbringer, P. 1911. Zur Kenntnis der Natur der Prostatakörner. *Zeitsch. f. Urol.*, **5**, 169.

Gegenbauer, C. 1899. *Lehrbuch der Anatomie des Menschen.* Leipzig: W. Englemann.

Gleisberg, J. P. 1886. *Die männlichen und weiblichen Geschlechtsorgane, deren Bau, Verrichtungen und Krankheiten.* Dresden: F. Tittel.

Griffiths, J. 1890. Observations on the function of the prostate gland in man and the lower animals. *J. Anat. and Physiol.*, **24**, 27.

Guelliot, O. 1883. *Des vésicules seminales, anatomie et pathologie.* Paris.

Hada, B. 1914–15. Studien zur Entwicklung, zur normalen und pathologischen Anatomie der Prostata, mit besonderer Berücksichtigung der sogennanten Prostata hypertrophie. *Folia Urol.*, **9**, 1.

Henle, J. 1863. Über die Cowperschen Drüsen. *Göttinger Nachrichten*, **13**.

Hermes, R. 1893. *Die Epithelverhältnisse in den Ausführungsgängen der männlichen Geschlechtsdrüsen.* Inaug. Dissert. Rostock.

Herxheimer, G. 1904. Fettinfiltration und Fettdegeneration. *Erg. d. allg. Path. u. path. Anat.*, **8**, 625.

Hyrtl, J. 1889. *Lehrbuch der Anatomie des Menschen.* Wien: W. Brausmüller.

Ishihara, M. 1915. Über das Lipoidpigment der Prostatadrüsen und über die Pigmente des Ductus ejaculatorius und der Prostatamuskulatur. *Folia Urol.*, **9**, 280.

Iwanoff, E. 1900. La fonction de la vésicule séminale et de la glande prostatique dans l'acte de la fecondation. *J. de Physiol. et de Path. Gén.*, **2**, 95.

Jacoby, M. 1923. Zur Prostatahypertrophie. *Zeit. f. urol. Chir.*, **14**, 6.

Jores, L. 1894. Über die Hypertrophie des sogenannten mittleren Lappens der Prostata. *Virchow's Archiv*, **135**, 224.

Kaiserling, C. 1910. Nachweis, Vorkommen und Bedeutung der Zelllipoide. *Berl. klin. Wochenschr.*, **47**, 2156.

Kawamura, R. 1911. *Die Cholesterinesterverfettung*. Ed. 4, Jena: G. Fischer.

Kayser, H. 1889. *Untersuchungen über die Bedeutung der Samenblasen*. Dissert. Berlin.

Keyes, E. L. 1923. *Urology; diseases of the urinary organs, diseases of the male genital organs; the venereal diseases*. New York and London: Appleton.

Kinoshita, M. 1920. Die Lipoide der Prostata. *Zeit. f. Urol.*, **14**, 145.

Kolster, R. 1902. Über einen eigenartigen Prozess in den Samenblasen von Cervus alces. *Arch. f. mikr. Anat.*, **60**, 100.

Krause, R. 1913. *A course in normal histology*. New York: Rebman Co. Translation by Schmahl.

Kunze, A. 1922. Das physiologische Vorkommen morphologisch darstellbarer Lipoide in Hoden und Prostata, mit besonderer Berücksichtigung der Haussäugetiere *Arch. f. mikr. Anat.*, **96**, 387.

Langerhans, P. 1874. Über die accessorischen Drüsen der Geschlechtsorgane. *Virchow's Archiv*, **61**, 208.

Leydig, F. 1850. Zur Anatomie der männlichen Geschlechtsorgane und Analdrüsen der Säugetiere. *Zeit. f. wiss. Zool.*, **2**, 1.

Limon, M. 1901. Note sur l'épithélium des vésicules séminales et de l'ampoule des canaux déférents du taurau. *J. d. l'anat. et physiol.*, **37**, 424.

Lode, A. 1896. Experimentelle Beiträge zur Physiologie der Semenblasen. *Sitzungsbericht d. kais. Akad. in Wien*, **104**,

Loeschke. 1920. Über Wesen und Entstehung der Prostatahypertrophie. *Münch. Med. Wochensch.*, **67**, 302.

Löwenthal, N. 1897. Note sur la structure fine des glandes de Cowper du rat blanc. *Bibl. anat.*, **4**, 168.

Lowsley, Oswald S. 1912. The development of the human prostate gland with reference to the development of other structures at the neck of the urinary bladder. *Am. J. Anat.*, **13**, 299.

Maass, F. 1889. Zur Kenntnis des körnigen Pigmentes im menschlichen Körper. *Arch. f. mikr. Anat.*, **34**, 452.

MacCallum, W. G. 1924. *A text-book of pathology*. Phila. and London: W. B. Saunders.

McMurrich, J. Playfair. 1914. Urinogenital system. In Morris's *Human Anatomy*. Ed. 5, Philadelphia: P. Blakiston's Son & Co. P. 1257.

Mansell-Moullin, C. 1895. A contribution to the morphology of the prostate. *J. Anat. and Physiol.*, **29**, 201.

Maziarsky, St. 1901. Über den Bau und die Einteilung der Drüsen. *Anat. Hefte*, **18**, 1.

Minot, C. S. 1885. Zur Kenntnis der Samenblasen beim Meerschweinchen. *Arch. f. mikr. Anat.*, **24**, 211.

Mislawsky, N., and Bormann, W. 1898. Die Sekretionsnerven der Prostata. *Centralb. f. Phys.*, **12**, 181.

Motz and Perearneau. 1905. Contribution à l'étude de l'évolution de l'hypertrophie de la prostate. *Ann. des maladies des organes génito-urinaires*, **23**, 1521.

Müller, V. 1892. Über die Entwicklungsgeschichte und feinere Anatomie der Bartholinischen und Cowpersehen Drüse des Menschen. *Arch. f. mikr. Anat.*, **39**, 33.

Munk, F. 1908. Über lipoid Degeneration. *Arch. f. path. Anat. u. Phys.*, **194**, 527.

Oberndorfer, S. 1902. Beiträge zur Anatomie und Pathologie der Samenblasen. *Ziegler's Beiträge*, **31**, 325.

Oppel, A. 1904*a*. *Lehrbuch der vergleichenden mikroskopischen Anatomie der Wirbeltiere.* IV. Jena: G. Fischer. Article on human prostate, p. 396.

——— 1904*b*. Article on seminal vesicle. *Ibid.*, 395.

——— 1904*c*. Article on Cowper's glands. *Ibid.*, 403.

Oudemans, J. Th. 1892. *Die accessorischen Geschlechtsdrüsen der Säugethiere.* Haarlem.

Pallin, G. 1901. Beiträge zur Anatomie und Embryologie der Prostata und der Samenblasen. *Arch. f. Anat. Anat. Abth.*, 135.

Petersen, O. V. C. E. 190-. Beiträge zur mikroskopischen Anatomie der Vesicula seminalis des Menschen und einiger Säugetiere. *Anat. Hefte*, **34**, 239.

——— 1909. Beiträge zur Histologie der Prostata. *Ibid.*, **39**, 655.

Plenge, C. 1924. Über Lipoide und Pigmente der Prostata des Menschen. *Archiv f. path. Anat. u. Phys.*, **253**, 665.

Pohl, H. 1923. Über das Epithel in den Samenblasen des Meerschweinchens. *Anat. Anz.*, **57**, 266.

Posner, C. 1912*a*. Die Bedeutung der Lipoide für die Urologie. *Verh. d. deuts. Ges. f. Urologie*, **3**, 179.

——— 1912*b*. Bemerkungen über das Prostatasekret. *Zeit. f. Urol.*, **5**, 161.

Posner, H. L. 1911. Prostata lipoid und Prostatakonkretionen. *Zeit. f. Urol.*, **5**, 722.

Pousargues, M. de. 1893. Détails anatomiques sur l'appareil génital mâle du Cavia cobaya. *Am. Sci. Naturel.* (*Zool. et Palaeont.*) (7 série.) **15**, 343.

Prenant and Bouin. 1910. *Traité d'histologie.* Paris: Masson et Cie. **2**.

Randisi, F. 1912. I Lipoide nella prostata. *Folia Urol.*, **6**, 588.

Rapoport, L. 1911–12. Prostatasekret. *Verh. d. deuts. Ges. f. Urol.*, **3**, 184.

Rauther, Max. 1903. Über den Genitalapparat einiger Nager u. Insektivoren insbesondere die accessorischen Genitaldrüsen derselben. *Janaische Zeit. f. Naturwis.*, **38**, 377.

Rehfisch, E. 1896. Neuere Untersuchungen über die Physiologie der Samenblasen. *Deut. med. Wochenschr.*, **22**, 245.

Remy Saint-Loup, M. 1894. Sur les vésicules séminales et l'uterus mâle des rongeurs. *C. R. de la Soc. de Biol.*, **46**, 1.

Rüdinger, N. 1883. Zur Anatomie der Prostata, des Uterus masculinus und der Ductus ejaculatorii beim Menschen. *München. Festschr. dem Ärztl. Verein, München.*

Schaap, P. C. D. 1899. *Die Glandulae genitales accessoriae des Kaninchens im normalen Zustande und ihre Veränderungen nach Kastration und nach Resektion der Vasa deferentia. Onderzoekingen gedan in het Physologisch Laboratorium der Utrechtsche Hoogeschool uitgegeven door C. A. Pekelharing en H. Zwaardemaker.* 1. Bd. Utrecht.

Schäfer, E. A. 1912. *Text-book of microscopic anatomy.* London and New York: Longmans, Green & Co.

Schaffer, J. 1922. *Lehrbuch der Histologie und Histogenesee.* Ed. 2, Leipzig: W. Englemann.

Schlagenhaufer, F. 1909. Über Lipoide, doppel brechende Substanzen in Prostatacarcinomen *Verh. d. dtsch. pathol. Ges.*, 332.

Schneidenmühl, H. 1883. *Vergleichend anatomische Untersuchungen über den feineren Bau der Cowperschen Drüse.* Inaug. Diss. Erlangen 28 s. Hanover.

Sehrt, E. 1913. Über das Vorkommen einer doppellichtbrechenden Substanz als normaler Bestandteil der Prostataepithelzelle des Menschen und Farren. *Arch. f. path. Anat. u. Phys.*, **214**, 132.

Steinach, E. 1894. Untersuchungen zur vergleichenden Physiologie der männlichen Geschlechtsorgane insbesondere der accessorischen Geschlechtsdrüsen. *Pflüger's Archiv*, **56**, 304.

Stilling, H. 1884. Beobachtungen über die Function der Prostata und über die Entstehungen der prostatischen Concremente. *Virchow's Archiv*, **98**, 1.

——— 1885. Über die Cowperschen Drüsen. *Ibid.*, **100**, 170.

Szymonowicz, Ladislaus. 1924. *Lehrbuch der Histologie.* Ed. 5, Leipzig: Curt Kabitzsch.

Timofeew, D. A. 1894. Zur Kenntnis der Nervenendigungen in den männlichen Geschlechtsorganen der Säuger. *Anat. Anz.*, **9**, 342.

— — 1895–96. Ueber eine besondere Art von eingekapselten Nervenendigungen in den männlichen Geschlechtsorganen bei Säugetieren. *Anat. Anz.*, **11**, 44.

Walker, G. 1899. Beiträge zur Kenntnis der Anatomie und Physiologie der Prostata, nebst Bermerkungen über den Vorgang der Ejakulation. *Arch. f. Anat. u. Entw.*, 313.

— — 1900. A contribution to the study of the anatomy and physiology of the prostate gland, and a few observations on the phenomenon of ejaculation. *Johns Hopkins Hospital Bull.*, **11**, 242.

——— 1910. A special function discovered in a glandular structure hitherto supposed to form a part of the prostate gland in rats and guinea-pigs. *Ibid.*, **21**, 182.

Warnock, A. W. 1923. The anatomy of the seminal vesicles of the guinea-pig, *Caria Cobaya*. *Anat. Record*, **25**, 275.

Weski, O. 1903. Beiträge zur Kenntnis des mikroskopischen Baues der menschlichen Prostata. *Anat. Hefte*, **21**, 63.

Young, Hugh H., and Davis, David M. 1926. *Young's practice of urology*. Philadelphia and London: W. B. Saunders Co.

Zimmermann, K. W. 1898. Beiträge zur Kenntnis einiger Drüsen und Epithelien. *Arch. f. mikr. Anat.*, **52**, 552.

PLATE I*

FIG. 1.—Epithelium of a depression of the mucous membrane of the seminal vesicle of an adult, in secretion. Note the masses of secretion adhering to the free surface of the cells. (After Eberth, 1904.)

FIG. 2.—Epithelium of the seminal vesicle of an executed criminal, showing the one-layered condition. (After Zimmermann.)

FIG. 3.—Same as Figure 2, but showing two layers. In both Figures 2 and 3 the cells show diplosomes and, in the surface cells, short flagella are present. (After Zimmermann.)

Note. Figs. 4 to 10 were redrawn from the figures of Akutsu (1903*b*.) They represent epithelial cells from the seminal vesicle of the rat.

FIG. 4.—Cells before development of secretion granules.

FIG. 5.—Early stage of secretion granule formation.

FIGS. 6 and 7.—Later stages in development of secretion granules.

FIG. 8.—Cells charged with secretion granules which retain their cylindrical form.

FIG. 9.—Fully charged epithelial cells before stimulation.

FIG. 10.—Epithelial cells after stimulation of the hypogastric nerve, showing the depletion in secretion granules. Compare with Figure 9.

FIG. 11.†—Mitochondria in seminal vesicle of white mouse. These are typically filamentous, although short rods and granules are also seen, and are basally situated. In the supranuclear zone, secretion granules occur in wide spaces. Mass of secretion in lumen, of similar staining properties to granules. (After Cowdry.)

*Consecutive figures 666–676.

†Figs. 12, 13, 14. For caption see second page following.

PLATE I

PLATE I, including Figures 1–14.—Epithelium of the seminal vesicles.

PLATE II*

Fig. 15. Epithelium of a glandular cul-de-sac of the human prostate. There is a superficial layer of cylindrical cells of variable height, which may be large and lightly staining, or narrow and dark. The basal cells are endotheliform. (After Prenant and Bouin, 1910.)

Note. Figures 16 to 20 are of the prostate of a twenty-four-year-old man, and show various stages in the formation and evacuation of secretion granules. (After Petersen.)

Fig. 16.—Shows the pre-granule condition, the cytoplasm being lightly staining, with a faint reticulum. (After Petersen.)

Fig. 17.—Shows an early stage in secretion-granule development. The cytoplasm is filled with large and small granules, of basophil reaction, which are sharply outlined. (After Petersen.)

Fig. 18.—Shows the cytoplasm filled with large and small granules, some of which are purely basophil, and some purely acidophil, while still others are composed of both materials. Often there is a basophil granule surrounded by an acidophil periphery, and sometimes the reverse is seen. (After Petersen.)

Fig. 19.—The reaction of the granules has now changed to acidophil, and they are ready to be discharged. The granules are now smaller than before. (After Petersen.)

Fig. 20.—Two cells are shown in process of delivery of secretion. The cell boundaries have lost their sharpness of outline, and there are two distinct zones in the cell, a peripheral, in which granules and an indefinite reticulum may be made out, and which often shows prolongation of the granule-holding cytoplasm into the lumen; and a basal, without granules, and in which the cytoplasm resembles that of Figure 16, showing the same type of lightly-staining reticular formation. A clump of granular secretion has become freed from the cell to the right. (After Petersen.)

Fig. 21.—Prostate of white mouse, containing very minute mitochondria. (After Cowdry.)

Fig. 22.—Prostate of a forty-two-year-old man, prepared to show lipoid, which is seen as numerous small black granules in the region of the nucleus. Small fat granules see also free in the lumen. (After Plenge, modified.)

Fig. 23.—Prostate of a forty-nine-year-old man, showing doubly refractive lipoid lying in the lumen as well as within the epithelial cells, as disclosed by polarized light. (After Plenge.)

*Consecutive figures 680-688.

Figures 12, 13 and 14* show stages in the process of manufacture and discharge of secretion in the seminal vesicle of a twenty-year-old man. (After Petersen.)

Fig. 12. Cell full of small acidophil granules, apparently the precursors of the secretion granules. Large acidophil secretion granules near the nucleus. (After Petersen.)

Fig. 13.—In the peripheral part of the cell are large isolated acidophil granules in a reticulum. The fine granular protoplasm is decreasing in amount. (After Petersen.)

Fig. 14.—Further decrease in small basal granules, while the larger peripheral granules have increased in size, and the cytoplasm is here filamentous or reticular. (After Petersen.)

*Consecutive figures 677–679.

PLATE II

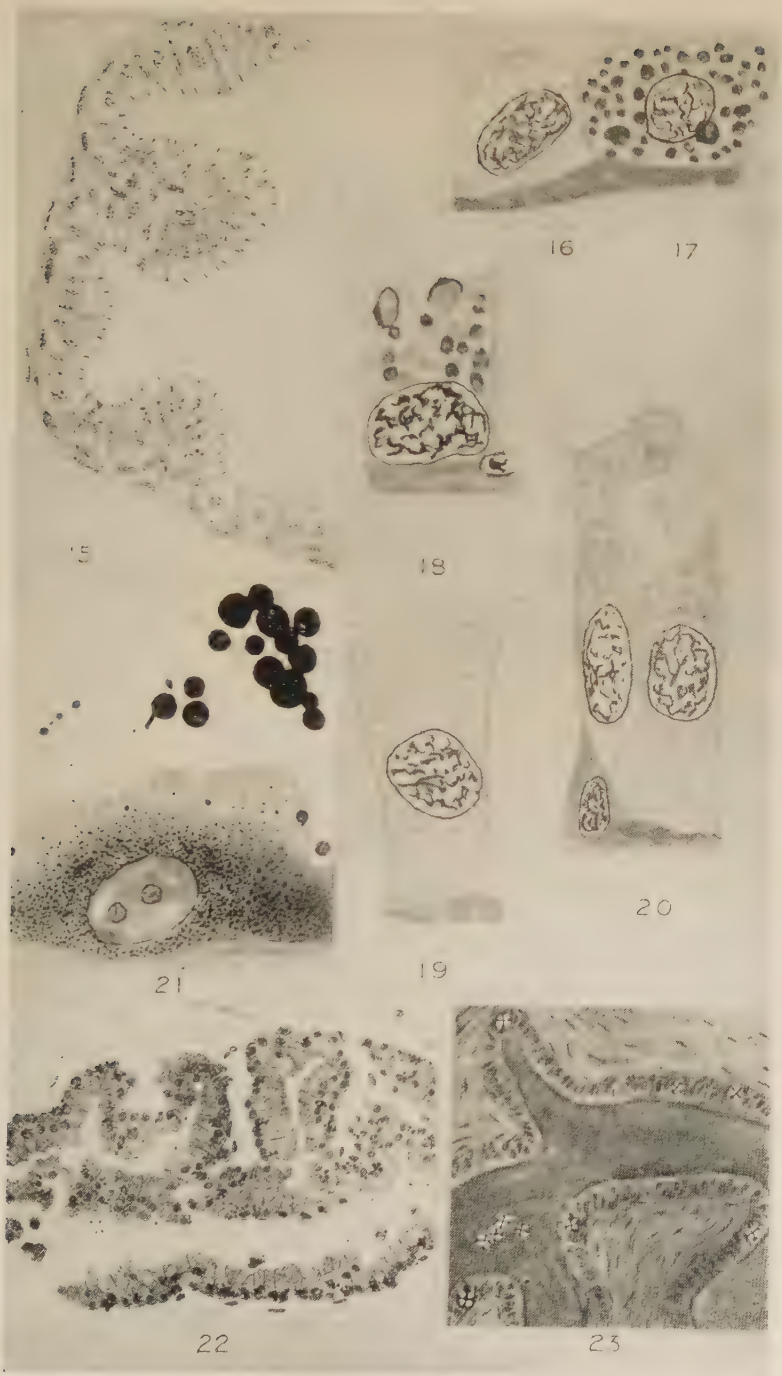


PLATE II, including Figures 15–23.—Epithelium of the prostate.

PLATE III*

FIG. 24.—Low-power view of an area of Cowper's gland from an executed man of twenty-one years, showing a glandular cul-de-sac cg and an ampullary dilatation (amp) of an excretory duct, bearing flattened epithelium. From Braus, after Prenant and Bouin.)

FIG. 25.—High-power view showing epithelium covering a an ampullary dilatation; (b) a connecting duct or stalk, and (c) an acinus. (From Eberth, after Braus.)

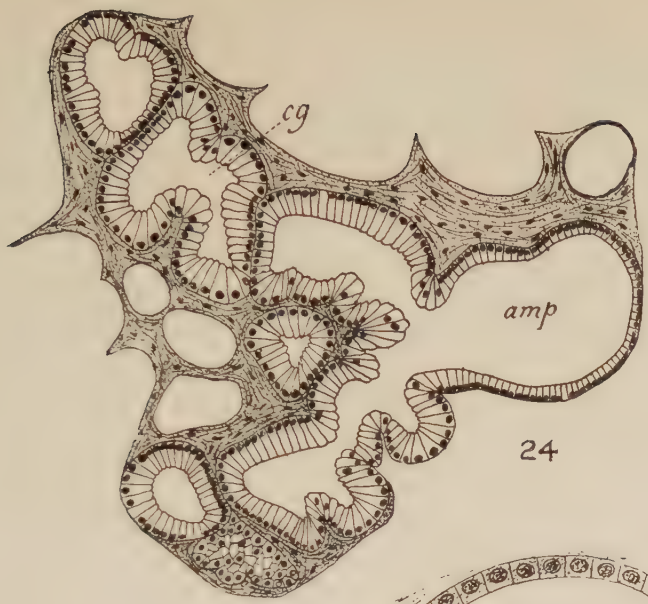
FIG. 26.—Acinus of Cowper's gland. Note spaces (secretory capillaries?) between the gland cells (a), and the reticular character of the cytoplasm, from which the secreting granules have been dissolved out. (From Eberth, after Braus.)

FIG. 27.—Epithelium of Cowper's gland, schematic, showing the quiescent stage. (From Eberth, after Stilling.)

FIG. 28.—Same as Figure 27, but after copulation, showing the widening of the lumen and thinning of the cells. (From Eberth, after Stilling.)

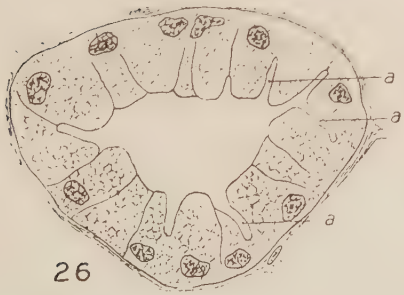
*Consecutive figures 689–693.

PLATE III

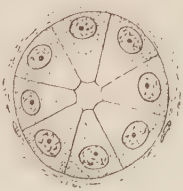


24

25



26



27

28

PLATE III, including Figures 24–28.—Epithelium of the bulbourethral glands.

INDEX

INDEX

This is the complete index to both volumes. It should be noted that pages 1–702 are in Volume I, pages 703 and following are in Volume II.

Absorption, fatty, 177; from joints, 751; iron, 178; protein, 178–180; water, 178
Accessory nasal sinuses. *See* Paranasal sinuses
Acervulus. *See* Brain sand
Acidophilic cells of pinial resembling those of parathyroids, 520
Acidosis, effect on erythrocytes, 280
Acinous cells of pancreas, 241–246, 254–257
Acroblast, 1283, 1288, 1292
Acromegaly, adenoma of, 497–498; thymic regulation in, 598; thyroid changes in, 565
Acrosome, 1283, 1288
Addison's disease, 30; pigmentation in, 640; thymic hyperplasia in, 602
Adenoids, 57
Adenoma of: hypophysis, 498; thyroid, 559
Adjustors, nervous, 16
Adrenal insufficiency, compensated for by corpus luteum, 650
Adrenalin, effect on retina, 912. *See also* Epinephrin
Adrenals. *See* Suprarenal bodies
Adventitial cells of Marchand, 427, 436. *See also* Macrophages
Age, effect on: atrophy of suprarenal, 649; bulbo-urethral glands, 1313, 1314; chromidial substance in sympathetic cells, 1013, 1014; epithelial cells of Fallopian tube, 1145; erythrocytes, 282, 284; form of cardiac muscle fiber, 813; hemopoiesis, 302; pancreas, 251; pigmentation of heart, 841; pigmentation of suprarenal, 641; pineal, 520, 523, 528–532; mammary glands, 1181–1184; testicular interstitial cells, 1224; thymus, 597; sympathetic cells, 1013, 1014, 1015, 1023
Agglutimins, relation to erythrocytes, 280
Albarran's glands, 1313
Albuminous glands, salivary, 93
Alcoholic intoxication, effect on interstitial cells, 1239
Aleucemic reticulosis, histiocytes in, 429
Aleuronoid granules in retinal epithelium, 903
Alimentary tract, protective mechanism in, 18
Alkaloids, function of liver in destruction of, 228
All or none law, nerve cells, 994
Allergic reaction, 18
Alpha cells of: hypophysis, 491–493; pancreas, 247, 266
Altitude, effect on erythrocytes, 284
Altmann's granules, in part mitochondria, 24
Alveolar epithelium, 78–85; cell types, 80
Alveolar pores, 85
Alveolar reticulum, 85
Alzheimer's Gitterzellen, 1058
Amblychromatic nuclei, 375
Ameboid motion: basophiles, 417; eosinophiles, 412; lymphocytes, 327; mammary gland epithelium, 1202; megalocaryocytes, 422; mesoglia, 1061; neutrophiles, 408–409; platelets, 418; small thymic cells, 594
Amenorrhea, changes in mammary stroma, 1201
Amitotic division: lymphocytes, 330, mammary gland, 1193
Amphitrope cells, 93
Amyloid degeneration, cardiac muscle, 846
Anal glands, 189
Anaphylactic shock and eosinophilia, 414
Anemia, pernicious, effect on erythrocytes, 282; sickle cell, 310
Anesthetics, effect on epinephrin store, 629
Angioblast, 297
Anisocyte, 276
Anoxemia, effect on erythrogenesis, 303
Antagonism between: mitosis and amitosis, 1194; secretion and mitosis, 1190
Antecedents of secretion, 141
Anterior gastric glands, 141
Anterior labial glands, 115
Antibody formation: by histiocytes, 440; by thymus and lymphoid tissues, 599
Antioxydase, 29
Antipressor substance of liver, 233
Antiprothrombin, 421
Aplasia, parathyroid, 576
Appendix, tumors of, 187
Aqueductus cochleae, 931
Aqueductus vestibuli, 931
Arachnoid membrane, 1082
Arachnoid villi, 1095–1099
Argentaffine cells. *See* Basal granular cells
Arneth formula, 405
Astroblast, 1045
Astrocytes: cytogenesis, 1044; function, 1064; gliosis, 1055; independence of, 1040; relation to blood vessels, pia and neurons, 1043–1044; structure, 1034–1039; types of, 1039

Atrio-ventricular bundle, 854ff.
Atrio-ventricular node, 853ff.
Auer bodies in myeloblasts, 380
Auricular fibrillation, Purkinje system in, 856
Autolysis, mechanism of, 1195, 1196
Autonomic nervous system, 1009
Autotransplantation, 1203
Avascular character of epidermis, 35
Axon hillock, 1016
Axons, 994ff.; medullated and non-medullated, functionally different, 994; sympathetic nerve cells, 1016–1018
Azurophile granules, 324, 326; in monocytes, 458; in myeloblasts, 379–380

Bactericidal function nasal mucous membrane, 65
Ballooning of intestinal cells, 173, 174, 179
Bartholin's gland, 123
Bartholin's major duct, 127
Basal cells of nasal mucous membrane, 52; respiratory, 72
Basal filaments, epidermal, 25
Basal filaments. *See* intracellular fibrils
Basal granular cells of intestine, 185
Basal membrane: olfactory, 56; paranasal sinuses, 62
Basement membrane of epidermis, 21; of bronchiole, 77
Basophile leucocytes, 415–417; centrosomes, 416; mitochondria, 416; number and size, 415
Basophilic stippling, 307
Beta cells of: hypophysis, 491–493; pancreas, 247, 266
Betz cells, 1065
Bile concentration, 224; pigment elaboration by histiocytes, 475; secretion, 224–226
Biliary channels, 220–222
Biliary system, intrahepatic, 221; extrahepatic, 222
Bilirubin, 225; excretion, 308; formation, 309
Binnennetz. *See* Golgi apparatus
Binucleate sympathetic cells, increase with age, 1014
Biochemistry of: smooth muscle, 798–799; striated muscle, 787–789
Bio-electric phenomena: erythrocytes, 288; nerve cells, 980
Bivalent chromosomes, 1274
Bizzozero, nodes of, 26
Blockade, histiocytic, 475
Blood, buffers in, 292
Blood coagulation, liver in, 229
Blood crisis, 306
Blood groups, 295, 308
Blood polyblasts and monocytes, 352; histiocytes, 455–458
Blood pressure after adrenalectomy, 632
Blood sugar, 226
Blood supply of: alveoli, 82; cartilage, 708; epidermis, 34; hypophysis, 490; kidney, 697–700; liver, 209; mammary gland, 1196; nose, 57–58; osseous and membraneous labyrinths, 930, 934, 940; parathyroids, 578; pineal, 532; salivary glands, 123; smooth muscle, 795; synovial membrane, 749; testis, 1241; thymus, 592; thyroid, 556
Blood transfusion, 294
Blood vessels, relation to astrocytes, 1043
Bodies of Call and Exner, 1129
Body chief cells of gastric glands, 148–155, 162
Body temperature and the suprarenals, 636
Boettcher's cells, 943
Bone, 712–733; bibliography, 733; calcification, 731; cell structure, 720–722; decalcified, 719–733; development membrane bones, 731; embryology, 727–730; growth 713; halisteresis, 732; Haversian systems, 715, 722; lacunae and canaliculi, 718, 721; lamellae, 715–718; lammellar structure, 718, 722; resorption, 712, 724, 727; undecalcified, 715–719; variations in cytology, 723
Bone marrow: effect of feeding on, 299; histiocytes of, 450–451; monocytes from, 459
Bone metabolism, 719
Boric acid, diffusion through skin, 15
Bowman's capsule, 668, 674
Bowman's glands, 52
Brain sand in pineal, 525, 532, 541
Branchiogenic derivatives, 553
Bronchial epithelium, 71–74
Bronchiolar epithelium, 74–77
Bronchioli respiratorii, 75ff.
Brown atrophy of heart, 830, 841
Brunner's glands. *See* Duodenal glands
Buccal glands, 123
Buffers: of blood, 292; surrounding muscles, 788, 789
Bulbo-urethral glands, 1313–1316
Burns, changes in chromidial substance after, 1025
Bursae. *See* Synovial membrane

Cabot's rings, 307
Cachexia strumipriva. *See* Myxedema
Calcareous concretions in pineal, 519, 527 532
Calcification, 731–732; cardiac muscle, 846
Calcium in: bone formation, 710; epidermis, 35; halisteresis, 732; relation to cardiac muscle, 846; tetany, 582
Calcium metastases, 847
Canalicular apparatus. *See* Golgi apparatus
Canaliculi of bone, 718, 721
Canalis cotunii, 931
Capillaries, in living conditions of dermis, 13; and blood formation, 299ff.
Captivity, effect on testicular interstitial cells, 1230

Carbohydrate, feeding, 185; influenced by insulin, 267; metabolism, 226, 263–268
Carbon dioxide of blood, effect on: erythrocytes, 288; retina, 912
Carbon dioxide, production by nerves, 980; transported by hemoglobin, 291
Carbon monoxide, resistance of skin to, 15
Carboxylase of skin, 17
Carcinoma: of mammary glands, 1203–1204; Paneth cells in, 185
Carcinomatous transformation of mammary glands, 1202
Cardiac cells formed from Purkinje cells, 882
Cardiac glands, 139
Cardiac impulses: carried by Purkinje system, 853; origin of, 856
Cardiac muscle, 807–851; amyloid degeneration, 846; bibliography, 847–851; calcification, 846; contraction and contraction band, 812, 834; cytogenesis, 815–820; cytology in relation to beginning contraction, 820–821; fatty degeneration, 845; fibrils, 808, 821; general description, 808–815; growth, 841–842; hypertrophy and regeneration, 843–845; innervation, 795; 1017, 1018; intercalated discs, 828–834; M line, 824; N and E discs, 826; physical and chemical changes in contraction, 835–837; Q and J discs, 824; refracting substance, 839; sarcolemma, 837–839; sarcosomes, 825, 827; sarcoplasm, 839; segmentation, 842; terminology, 809; transverse striations, 822–827; Z line, 823
Carotin, in lutein tissue of cow, 1137
Carotinoid pigmentation of skin, 28
Cartilage, 705–712; bibliography, 733; blood supply, 708; cartilage cell, 706; cellular changes, 709–711; function, 707; hyaline, 705; matrix, 706; perichondrium, 705; transition to synovial membrane, 748–749; types, 705; white fibrous, 711; yellow elastic, 711; innervation, 709
Casein, production of, 1192
Castration effect on: bulbo-urethral glands, 1314; mammary gland, 1183, 1195, 1198; suprarenals, 651
Castration, partial, effect on interstitial cells, 1234
Catarrhal states, 64
Cell capsules: proliferation of, 1025; of sympathetic nerve cells, 1019
Cell division, influenced by oxygen supply, 1193
Cell growth, mechanism of, 5
Cell injury: detection of, 6; electrical productivity in, 6
Cell respiration, 6
Cell structure, influence of medium on, 3
Cell surface in relation to volume, 8
Cells and metazoa contrasted, 3
Cellular reactions, cinematographic analysis of, 6, 8
Cement substance, intestinal cells, 173, 178
Centrifuging nerve cells, 967
Centriole: gastric glands, 146; histiocytes (macrophages), 432; lymphocytes, 326; plasma cells, 356; testicular interstitial cells, 1215
Centroacinous cells of pancreas, 251–253
Centrosomes: absent in microglia, 1037; in astrocytes and oligodendroglia, 1036; in epidermal cells, 31; in gastric cells, 146; in intestinal cells, 175; in nerve cells, 977; in neutrophilic leucocytes, 406
Centrosomes of: basophiles, 416; male germ cells, 1283ff.
Cephalin of erythrocytes, 290
Cerebrosids of erythrocytes, 290
Cerebrospinal fluid, 1076–1077, 1086; absorption of, 1095–1099; cells in, 1089–1094; lymphatic drainage, 1099–1102; physiologically active substances in, 1077–1078
Cerebrospinal pathway, 1071–1107; arachnoid villi, 1095–1099; bibliography, 1102–1107; cerebral ventricles, 1071–1078; development of meningeal spaces, 1083–1085; leptomeninges, 1082–1095; meninges, 1080–1083; nerve roots, 1099–1102; perivascular and perineuronal spaces, 1086–1089; roof fourth ventricle, 1078–1080; subdural space, 1085
Cervical sympathetic ganglion (superior), 1022
Chemistry of: erythrocytes, 289–290; lipoids, 647–648; nasal mucous membrane, 65; thyroxin, 562–563
Chief cells of parathyroids, 579
Chloride shift in blood, 291
Chlorides of blood, effect on erythrocytes, 288
Cholesterin in suprarenals, 647
Cholesterinogenic function, 651
Cholesterol: of erythrocytes, 290; in liver, 225
Cholesterol in histiocytes, 475
Choline and nerve cell injury, 983; in prostatic secretion, 1309
Chondrioconts. *See* Mitochondria
Chondriomites. *See* Mitochondria
Chondriosomes. *See* Mitochondria
Chorda tympani: composition of, 116; stimulation of, 114
Chorioid plexuses: assumption of function by, 1079; origin, 1072–1074, 1083, 1084; secretion by, 1075; structure, 1074
Chromaffin cells of intestine, 185
Chromaffin cells. *See* Basal granular cells
Chromaffin granules of suprarenal, 625
Chromaffin inclusions of suprarenals, 625; total disappearance of, 648
Chromatin diminution, 1260
Chromatolysis of: granulosa cells, 1130–1131; nerve cells, 971
Chromatolysis by margination, 1131
Chromatophores. *See* Langerhans cells

Chromidial hypothesis, 100
Chromidial substance: in eggs, 1121; nerve cells, 968–971, 982; (Nissl substance) different types of neurons, 999–1000; pancreas, 243, 252; synovial membrane, 745; sympathetic nerve cells, 1011–1014, 1020, 1024
Chromogens, 29
Chromophile cells of hypophysis, 491
Chromophile cells of suprarenals, 625
Chromophile material, salivary glands, 99–102
Chromophile reaction, effect of temperature on, 632
Chromophile substance. *See* Chromidial substance
Chromophile system, 626, 632
Chromophobe cells of hypophysis, 491
Chromosomes, 1273–1282; chromosome behavior in diptera, 1274; growth stages, 1275, 1282; of man and mammals, 1282; terminology, 1274
Ciliary ganglion, 1020
Ciliary glands. *See* Glands of Moll
Ciliated cells: Fallopian tube, 1143; nasal, 55; respiratory, 72; of thyroid, 551
Cinematograph in cytology, 800
Circumanal glands, 36
Circumpolar bodies of Long and Mark, 1124
Clasmatocytes of Ranvier, introduction of term, 427, 435. *See* Macrophages
Clasmatocytoid histiocytes, 473
Clasmatodendrosis, 1054
Clasmatosis, 435
Claudius' cells, 943
Cochlea, 929ff.
Cohnheim's fields, 839
Coitus, effect on intranasal erectile tissue, 58
Colloid cells of thyroid, 558
Colloid in: epidermis, 35; hypophysis, 494, 497; thyroid, 559, 630; parathyroids, 580
Color index of erythrocytes, 288
Color vision, 911–912
Colostrum, 1190, 1194
Common bile duct, obstruction of, 223
Comparative anatomy of: erythrocytes, 283; gastric glands, 139; interstitial cells of testis, 1212–1214; liver, 206; meninges, 1080–1081; parathyroids, 576; thyroid, 551–552
Concha, inferior nasal, 55
Concha, superior nasal, 57
Conduction in medullated and non-medullated axons contrasted, 994
Connective tissue, histiocytes of, 429–436
Contraction and contraction band of cardiac muscle, 834
Contraction beginning, cytology of, in cardiac muscle, 820–821
Coordination, defined, 993; neurons, 1001–1004
Cornea, literature on, 17
Cornea of epiphyseal complex, 511
Cornification. *See* Keratinization, 13
Corpora amylacea, reference to, 978
Corpora cavernosa, nasal, 58
Corpora lutea, extirpation of, during pregnancy, 1177, 1188
Corpus luteum: effect in adrenalectomized animals, 650; effect on proliferation of mammary gland, 1185, 1187, 1188; effect on uterine mucosa, 1176; hormone of, 3, 1138; prevents ovulation, 1176; 1177. *See* also lutein cells
Corpus luteum produces several hormones, 1177
Correlation, defined, 993; neurons, 1001–1004
Cortex of suprarenals, 640–653
Corti's membrane. *See* Membrana tectoria
Corti's organ, 929ff.
Corti, tunnel of, 945
Coughing, effect on respiratory epithelium, 74
Counting, erythrocytes, 284
Cowper's glands. *See* Bulbo-urethral glands
Cretinism, 566
Crista spiralis, 936
Cross-breeding, effect on testicles, 1241
Cryptorchidism, effect on: testicular interstitial cells, 1230; spermatogenesis, 1294–1295
Crypts of Lieberkuehn, 171, 188
Crystalloids in: ova, 1120; testicular interstitial cells, 1221, 1232
Cumulus oophorus, 1114, 1129
Cutaneous glands, 36
Cuticle, interstitial, 175
Cystic duct, 221
Cysts of pars intermedia, 494
Cytocentrum of lymphocytes, 326
Cytoneural junction, effect of thyroid hormone at, 564
Cytological technique, 4
Cytology, necessary equipment for, 8; preparation for study of, 8; purpose of, 3, 4; relation to other sciences, 3, 9
Cytology, organization of laboratory for, 9; scope of, 4

Deaminization, 227
Death changes in cells, 8
Dedifferentiation, of intestinal cells, 181; of granulocytes, 390; of pancreatic cells, 262, 264
Defects, developmental thyroid, 554
Degeneration, hydropic: fatty, pancreas, 258; hyaline, pancreas, 259; pancreas, 259, 264; physiological, 258
Deiters' cells of internal ear, 944ff. *See* also Astrocytes
Delomorph cells of Rollett, 140
Demilunes of Heidenhain: nasal, 55; submaxillary, 104–107
Dendrite cells. *See* Langerhans cells
Dendrites, 994ff.; sympathetic nerve cells, 1018

Dendrophagocytosis, 1054, 1065
Depressor substance from liver, 232
Dermal chromatophores, 912
Dermis, relation to epidermis, 21
Destruction of erythrocytes, 309–313
Detoxicating action of liver, 228
Detoxifying action, eosinophiles, 415
Development of: cardiac muscle, 815–820; erythrocytes, 296–309; hypophysis, 489; macrophages or histiocytes, 463–470; mammary glands, 1180–1184; membrane bones, 731; meningeal spaces, 1083–1085; pancreas, 253; perilymphatic spaces, 932; renal tubules, 663, 665, 670; salivary glands, 115; of visual cells, 899
Developmental defects, thyroid, 554
Diabetes, effect on erythrocytes, 290
Diabetes mellitus and Islands of Langerhans, 263–264
Diakinesis, or prophase of chromosomes, 1278
Diastase of skin, 17
Diestrum, 1159, 1162
Diet, effect on: bones, 720; heart, 846
Diets, deficiency, effect on testicles, 1238
Diffuse or confused stage of chromosomes, 1278
Diffusion in nervous activity, 999
Diphtheria, regeneration of cardiac muscle in, 845
Diplotene stage of chromosomes, 1278
Diuresis and pineal extracts, 538
Donnan equilibrium, 291
Dopareaction of Bloch, 30
Duct ligation, effect on pancreas, 262, 264
Ducts: as contributors of water, 114; Bartholin's major, 127; of Luschka, 222; Ravinian, 129; salivary glands, 113–115; Stenson's, 127; Wharton's, 127
Ductus cochlearis, 932–934
Ductus endolymphaticus, 931
Duodenal glands, 187–189
Duplicity theory of retinal adaptations, 908 911
Dura, 1081
Dust cells of lung, 455
Dwarfism, 569

E discs of cardiac muscle, 826
Ear, internal, 929–961; bibliography, 959–961; membranous labyrinth, 934–945; membranous structures of vestibule and semicircular canals, 953–959; osseous capsule of labyrinth, 929–932; perilymphatic spaces, 932–934; supporting cells of papilla spiralis and lamina reticularis, 945–953
Ectopic testicles. *See* Testicles, nondescended
Edema, alveolar, 82; mechanical, of mitral stenosis, 85
Electrical phenomena of: nerves, 978, 980; retina, 905; skin, 15
Eleidin. *See* Keratohyalin
Embryo, effect on corpus luteum, 1176
Embryology: bone, 727–731; liver, 206; parathyroids, 575; renal tubules, 663, 670–674; synovial membrane, 737; thymus, 588–591; thyroid, 552–554
Emotions, effect on skin, 18
End cells, leucocytes as, 403
Endocarditis lenta, macrophages in, 457
Endocytes of liver capillaries, 452
Endolymph: impulses transmitted to, 949; movements of, 957; secretion of, 940, 942
Endomysium, 870, 871, 884
Endosteum, 730
Endothelial leucocytes, introduction of term, 459
Endothelial phagocytes, 447
Endothelium and blood: and reticular cells, 448; formation, 297; ingestion of particulate matter, 448; in tissue cultures, 448, 449
Enteric plexus, 1020, 1022
Environment: adjustment to, 965; temperature effect on testicles, 1235
Enzymes: and action of filterable viruses, 977; and pigmentation, 29; of erythrocytes, 290; of histiocytes, 475; of leucocytes, 353; of myeloblasts, 380, 381; of skin, 17, 35
Eosinophile cells of thymus, 596
Eosinophile leucocytes, 411–415; function, 414–415; number and size, 411; structure, 411–414
Eosinophilia, 413, 414
Eosinophilic myelocytes in cultures, 348
Ependymal cells of hypophysis, 494, 495
Epidermal fibrils, 25. *See* also intra-cellular fibrils
Epidermis, basement membrane of, 21
Epidermis, cytology of: avascular character, 35; calcium, 35; centrosomes, 31; colloid, 35; fatty substances, 34; ferments, 35; glycogen, 35; Golgi apparatus, 25; hemosiderin, 35; intercellular bridges, 26; intracellular fibrils, 25; keratin, 33; keratohyalin, 32; Langerhans cells, 32; lipochrome, 28; melanin, 27–32; mitochondria, 24; mucin, 35; nerve and vascular supply, 34; nuclei, 24; potassium, 35; physical consistency, 23; shape of cells, 22
Epidermis, mechanical protection, 13; chemical and physical properties, 14; hydrogen ion concentration 14; Keratinization, 13; protection against extremes of temperature, 16; protection against fluids and gases, 15; protection against light, 15
Epidermis, polarity of cells, 31
Epidermis, relation to dermis, 21
Epidermis, selective sensibility, 16; influenced by internal organs, 18; influenced by light, 17; influence of internal tissues, 17; internal secretion of, 17; sense organs, 16
Epimysium, 884

Epinephrin: amount, 627; chromium reaction, 626; discharge, 628, effect of denervation, 629; inclusions, 631–640; increased by thyroid feeding, 564; influence of infections on, 639; ratio to body weight, 627; thyroparathyroidectomy, 631
Epinephrin test in exophthalmic goiter, 564
Epinephrin test of Goetsch, 564
Epiphyseo-diaphysial line, 707
Epiphysis. *See* Pineal
Epithelioid cells, formation of, 352
Epithelioid cells of ovary, 1141–1142
Epithelioid reticular cells, 447
Epithelioma of hypophysis, 498
Epithelium: intestinal, 171–189; lower respiratory tract, 71–86; nasal respiratory, 54; paranasal, 61
Epsilon granules, 407
Erectility of generative organs and pineal extracts, 538
Erectility of nasal mucous membrane, 59, 65
Ergastidia of Laguesse, 99
Ergastoplasm of Garnier, 99, 141, 151
Erythroblast, 276
Erythroblast, primitive, 276
Erythrocyte, 276
Erythrocyte, primitive, 276
Erythrocytes, 275–318
  bibliography, 313–318
  chemistry, 289–290; ferments, glucose, hematin, 290
  fate, 309–313: disposal, 310; duration of life, 309
  function, 291–292; buffers of blood, 292; chloride shift; 291; oxygen tension, 291; permeability, 291
  hemolysis, 292–296: fragility, 292; reversible, 296; transfusion, 294
  origin, 296–309: erythrogenesis, 302; ontogenesis, 305; theories, 297
  physical characters, 277–298: hemoglobin, 286; number, 283; shape, 277; size, 280, 293; structure, 279; surface, 280; surface and volume, 287; suspension stability; 288, terminology, 276
Erythrogenesis, 299ff.
Erythrophagocytosis, 447
Erythropoiesis, 302; stimulus to, 302, 303
Esophageal glands, 142
Estrous cycle, 1169–1170; mammary glands during, 1185–1186, 1189; mammary secretion, 1189
Eunuchoidism, effect on interstitial cells, 1231
Excretion: by skin, 15; by kidney, 609
Exercise, effect on: body temperature, 632; erythrocytes, 281, 309
Exocrine glands, 36
Exophthalmic goiter, 566–569; nervous system in, 983
Exoplasmic fibers. *See* Intercellular bridges
Exteroceptors of Sherrington, 16, 992
Extracts of liver, 232
Exudate: serous, alveolar, 83; pneumonic, 81, 84; synovial, 752

Fallopian tubes, epithelium of: bibliography, 1150; cilia, 1143; cyclic changes, 1143–1145
Fasting, effect on glycogen: effect on pancreas, 256–257; effect on suprarenals, 651; in gastric cells, 146, 156
Fat absorption, 177
Fat metabolism, 227; histiocytes in, 475
Fatigue and the suprarenals, 632
Fatigue: effect on nerve cell, 970, 971, 981; central, its relation to cell body and dendrites, 995–998; effect on sympathetic cells, 1013
Fatty degeneration: cardiac muscle, 845; pancreas, 258; Purkinje cells, 884
Fatty substances: in epidermis, 34; in gastric glands, 142; in histiocytes (macrophages), 432, 445; lutein cells, 1134; mammary gland, 1191–1193; mesoglia, 1062; in nerve cells, 976; in neutrophilic leucocytes, 408; in pancreas, 245, 250; in retinal epithelium, 903; in suprarenals, 640, 647; in synovial membrane, 744
Feeding, effect on: blood formation, 298–299; bones, 719, 920; liver cells, 216; pancreas, 256; pineal, 537-538; testicles, 1236–1239
Fenestra tympani, 930
Fenestra vestibuli, 930
Ferments. *See* Enzymes
Fertilization, conditions favoring, 1159
Fetuses, effect of removal from uterus on mammary gland, 1188
Fibrillation of auricles, Purkinje system in, 856
Fibrils of: cardiac muscle, 808–810, 821; epidermis, 25; muscle, 772; nerve cells, 971–974; neuroglia cells, 1039; Purkinje cells, 865–870; of rods and cones, 897–899
Fibrinogen, relation of liver to, 229
Fibrinolytic power of mammary gland epithelium, 1202
Fibroblasts: change to histiocytes, 434; developed from lymphocytes, 434; distinguished from resting wandering cells, 427, 432; relation to polyblasts, 358
Fibrocartilage, 738
Filterable viruses: affecting epidermis, 35; affecting nerve cells, 977
Fingerprinting, 21
Flagellated thyroid cells, 551
Flagellum of spermatid, 1288; of spermatozoon, 1292ff.
Florescence of tissues, 7
Fluids, protection of epidermis against, 15
Focal necrosis, pancreas, 259
Follicles, thyroid, 557
Follicular fluid effect on uterus and vagina, 1177

Follicular hormone, effect on mammary glands, 1183
Foramen of Magendie, 1078
Foramina of Luschka, 1078
Fowl-pox, 25
Fractures of bone, healing of, 729
Fractures of cardiac muscle fibers, 842
Fragility, erythrocytes, 292
Fragmentation of cardiac muscle fibers, 842
Free-martin, interstitial cells in, 1242
Froehlich's disease, 569
Frontal sinus, 60
Fuchsinophile granules, in part mitochondria, 24
Fundus glands, 139
Fuscin in retinal epithelium, 901

Galactogogue effect, 18
Gall bladder: absence of, 222; effect of diet, 224; effect of fasting on, 222; function of, 223; wall of, 222–223
Gases: penetration of, 15; protection of epidermis against, 15
Gastric glands, 139–162
  cell types: body chief, 148–155; Heidenhain, 160–161; neck chief, 155–157; parietal, 157–160; Nüssbaum and Stohr, 161
  intestinal element, 161
  phylogenesis, 139
  reproduction of cells, 161
  surface epithelium of stomach, 141–147
Gastroenterostomy, effect on gastric glands, 162
Gaucher's disease, histiocytes in, 429, 448
Gelatin in cartilage matrix, 706
Genes, defined, 1261ff.
Genital organs and pinealectomy, 540–541
Germ cell determinants, 1260ff.
Germ cell, female. *See* Ovum
Germ cell, male, 1259–1300; bibliography, 1297–1300; centrosomes, Golgi bodies and idiozome material, 1283ff.; maturation, 1270–1292; mitochondria, 1284ff.; modifications, 1294–1297; origin, 1259–1270; products, 1270; spermatozoa, 1292–1294
Germ centers and lymphocyte formation, 330–341; active phase, 332; new active phase, 336; resting phase, 336; transition, 338
Gianuzzi's crescents: nasal mucous membrane, 59; salivary glands, 115
Gigantism and the pineal, 537
Gigantoblast, 276
Gitterzellen of Alzheimer, 1058
Gland: *See* anterior labial, Bartholins, buccal, labial, lesser sublingual, molar, parotid, palatine, retrolingual, salivary, sublingual, submaxillary
Glands: Albarran's, 1313; anal, 189; anterior gastric, 141; bulbo-urethral, 1313–1316; cardiac, 139; corpus luteum, *See* lutein cells; Cowper's, 1313–1316; duodenal, 187–189; esophageal, 142; exocrine, 36; fundus, 139; holocrine, 36; hypophyseal, 487–499; interrenal, 624; lateralis nasi, 60; mammary; 1175–1208; merocrine, 36; of Moll, 36; myometrial, 1188; neurocrine, 187; olfactory mucous membrane, 52; paranasal, 63; pineal, 503–548; proper gastric, 139; pyloric, 139; respiratory mucous membrane, 59; seminal vesicles, 1303–1308; Steno's, 60; sympaticotropen, or neurocrine, 187; testicular interstitial, 1211
Glandula lateralis nasi, 60
Gliosis of astrocytes, 1055
Gliosomes, 1038
Glisson's capsule, 209
Glomeruli in living condition, 5. *See* Renal glomeruli
Glucose: in carbohydrate metabolism, 226; in erythrocytes, 290; in tumor formation, 1203
Glycogen, formation of: in epidermis, 35; in gastric cells, 146; in liver reduced by thyroid feeding, 565; in nerve cells, 976; in neutrophilic leucocytes, 408; in tumors, 1232
Glycogen: in liver, 213, 226; in Purkinje cells, 861; and retina, 895
Glycolase of erythrocytes, 290
Glycosuria and pancreatic lesions, 263–264; and the thyroid, 565
Goblet cells: bronchial epithelium, 72; intestinal epithelium, 181–183; nasal mucous membrane, 55
Goiter, exophthalmic, 566–569; nervous system in, 983
Goiter, simple, 566, 568
Golgi apparatus: of astrocytes, 1037; of cerebrospinal nerve cells, 974; in gastric glands, 144–145, 153; in goblet cells, 183; in granulosa cell, 1128; of histiocytes (macrophages), 432; in intestinal cells, 176; in lutein cells, 1132–1134; of lymphocytes, 326; in male germ cells, 1283ff.; in ova, 1117–1118, 1122; in pancreas, 244, 250; of parathyroids, 580; in salivary mucous cells, 97; in skin, 25, 31, 32; of striated muscle, 783; of synovial membrane, 745; in testicular interstitial cells, 1222; of thyroid, 559
Golgi apparatus: reversal of polarity, 559, 580; rôle in fat absorption, 177
Gonadectomy, delaying thymus involution, 600; before puberty, 603
Gonial telphase of chromosomes, 1275
Graafian follicle, time of rupture, 1157
Grafts, testicular, interstitial cells in, 1234; thyroid, 564
Granular leucocytes. *See* leucocytes, granular
Granulocytes, 351
Granuloplasma, 356
Graves' disease, regeneration of thymus in; 598, 603
Growth factors, 1198
Growth inhibiting substances, 1187
Growth of cardiac muscle, 841–842

Growth rhythms of mammary gland, 1183
Growth stimuli, summation of, 1201
Guanidine, destroyed by liver, 233
Guanidine poisoning, 582, 583
Guanin in retinal epithelium, 902, 914
Gull's disease. *See* myxedema

H line in muscle, 772
Hair, 37
Hair cells of internal ear, 945ff.; stimulation of, 938
Hair glands, 36
Hairs, function of, 16
Halisteresis, 732
Haploid groups, 1273
Harderian gland, 17, 36
Hassall's corpuscles, 594
Haversian system, 715, 722
Hearing, sensation of, 938
Heart. *See* Cardiac muscle of. *See* Purkinje system of
Heat regulating mechanism: thyroids, 631; suprarenals, 636
Heidenhain cells, 140, 160
Heidenhain's demilunes, nasal mucous membrane, 59
Heinz bodies, 309
Heisterian valves, 221
Helicotrema, 934
Hemagglutinius, 311
Hematin, 290, 309
Hematoblast, 276, 417
Hematoidin, 309
Hematopoiesis: dualist theory, 342, 346; unitarian theory, 341, 344
Hematoporphyrin, 290
Hemoblast, lymphoid relation to myeloblast, 372
Hemochromogen, 290
Hemocytoblast, 276, 345, 348; relation to myeloblasts, 372, 385
Hemoglobin, 286; chemistry, 289
Hemoglobin, relation to granules of eosinophiles, 413; chemistry of, 289
Hemoglobinuria, paroxysmal, 310
Hemohistioblast, 276, 388; and blood histiocytes, 458
Hemohistiocytes, 387
Hemolysins, 295
Hemolysis, 292–296; reversible, 296
Hemolytic jaundice, effect on erythrocytes, 282, 294, 306; test for, 293
Hemophilia and platelets, 421
Hemopoiesis, 302
Hemorrhage, effect on erythrogenesis, 302, 303
Hemosiderin in epidermis, 35; in paranasal sinuses, 63; in tissues, 309
Hensen's cells, 944ff.
Heparin, isolated from liver, 232; in blood coagulation, 421
Hepatic extracts, 232
Hepatic lobule, 207ff.
Hepatopancreas, 206
Hermaphroditism, effect on testicular interstitial cells, 1231
Herpes, nuclear inclusions in, 35
Herpetic encephalitis, mitochondria in, 976
Hertwig's law of nucleus-plasma ratio, 1015
Herxheimer fibirls, 25
Herzfehlerzellen of lung, 455
Heterotransplantation of thyroid, 564
Hibernation in relation to fat formation, 228; influence of hypophysis, 497; influence on testicular interstitial cells, 1229
His, bundle of, 853ff.
Histiocytes of Kiyono, 428. *See* Macrophages
Histiocytes, showers of, in blood of right ventricle, 456
Histioid wandering cells, 464
Hodgkin's disease: eosinophiles in, 414; platelets increased in, 419; megalocaryocytes increased in lungs, 422
Holocrine glands, 36
Homeochrome cells, 93, 108
Homoio-transplantation, 1202
Hormones: corpus luteum, 3; epidermis, 17; ovarian, 1169; testicle, 3, 1241, 1242
Howell-Jolly bodies, 306
Hyaline cartilage, 705
Hyaline degeneration, pancreas, 259, 263
Hyaline material of hypophysis, 494, 497
Hydrocephalus, 1075, 1077
Hydrocyanic acid gas absorbed by skin, 15
Hydrogen ion concentration, 7; effect on erythrocytes, 278, 280; epidermis, 14; of joint exudates, 752
Hydrogen sulphide gas absorbed by skin, 15
Hydropic degeneration, pancreas, 259, 264
Hyperglycemia and pancreatic lesions, 265
Hypertrophy, islands of Langerhans, 260; cardiac muscle, 843–845
Hypophysis cerebri, 487–499; bibliography, 498–499; cellular pathology, 497–498; development, 489; microscopic structure, 491–496; primary subdivisions, 487–489; secretion, 497; vascular, lymphatic and nerve supply, 490
Hypophysis, influence on testicles, 1240
Hysterectomy, effect on corpus luteum and mammary gland, 1177

Idiozome, 117; of testicular interstitial cells, 1215
Idiozome material of male germ cells, 1283ff.
Immune bodies of skin, 17
Inanition effect on: liver cells, 216; lymphocytes, 353; pancreatic cells, 256, 258; parathyroids and suprarenals, 584; testicles, 1237; thymus cells, 321
Infection, resistance to, 639
Inflammation, 4; Kupffer cells in, 454; tuberculous, of liver, 452

Inflammation, 351; "aseptic" endothelium in, 471; formation of fibroblasts from reticular stroma, 450; "physiological" of Rössle, 440; polyblasts in, 439; sedimentation rate in, 289
Inflammation and changes in sympathetic cells, 1025; in estrous cycle, 1169
Inflammation, physiological; omentum, 440; during estrous and ovulation, 1169
Ingestion of particulate matter by endothelium, 448
Inheritance of skin color, 29
Inophragma of Heidenhain, 824
Insulin, effect on carbohydrate metabolism, 268
Insulin from: intestinal mucosa, 187, 193; pancreas, 268
Integration in nervous activity, 965, 999
Intercalated discs of muscle, 828–834, 877
Intercellular bridges, intestinal cells, 174; gastric cells, 142
Intercellular spaces: intestinal epithelium, 174–176; epidermis, 35
Interfacial tension method, 279, 280
Intermenstrual fluid, 1167
Internal secretions of: ovary, 1169; skin, 17
Internal tissues influenced by epidermis, 17
Interoceptive sensation, 992
Interoceptors of Sherrington, 16
Interrenal glands, 624
Intersexuality, 1231
Interstitial cells of brain, 1064
Interstitial cells of ovary: considered as histiocytes, 429; definition of term, 1141; properties, 1142–1143
Interstitial cells of testis, 1211–1356; abnormalities, 1230–1232; bibliography, 1243–1256; in captivity, 1230; cytomorphosis, 1223–1226; distribution in animal series, 1212–1214; experimental modifications, 1232–1241; finer structure, 1215–1222; functional significance, 1241–1243; general characters, 1211; hibernation, 1229; origin, 1222; seasonal variations, 1226–1229; in various diseases, 1231
Intestinal epithelium, 171–201; bibliography, 189–195; basal granular cells, 185–187; columnar absorbing cells, 172–180; duodenal gland cells, 187–189; goblet cells, 181–183; Paneth cells, 183–185; principal cells of crypts, 180; transitional epithelium, 189
Intestinal tract, protective mechanism in, 18, 171
Intestinal villus, 172
Intestine, tumors of, 187
Intracellular fibrils of skin, 25
Intracellular network. *See* Golgi apparatus
Intracellular spaces in liver cells, 209
Intranuclear inclusions, 35
Intravital dye storage, a physical phenomenon, 433
Involution, accidental, of thymus, 601
Involution with age, thymus, 597
Involution of thyroid influenced by iodine, 562
Iodine dosage, effect on thyroid, 567
Iodine, relation to thyroid structure, 561
Iodine in thyroid, 561–563
Iodophilia of neurophilic leucocytes, 408
Iodothyrin, 561
Iron absorption, 178; metabolism, 230, 414
Iron metabolism in histiocytes, 475
Irritability: of muscle, 786, 789, 794, 796; of nerve cells, 965, 991
Irritation, effect on respiratory epithelium, 74
Islands of Langerhans. *See* Langerhans islands

J discs in: cardiac muscle, 824; striated muscle, 772
J granules, 827
Jacobson's organ, 60
Jaundice, 223
Jaundice, hemolytic, effect on erythrocytes, 282
Joints. *See* Synovial membrane
Jolly bodies, 308

Kala azar, histiocytes in, 429
Keratin, 33
Keratinization, 33; oxygen supply, vitamin A, serum lipoids, 33
Keratoeleidin. *See* Keratohyalin
Keratohyalin, 32
Keratohyalin layer. *See* Stratum granulosum, 20
Kidney, gross anatomy, 695–697. *See also* Renal tubules
Krause's corpuscles, 16
Kultschitzky cells. *See* Basal granular cells
Kupffer cells of liver, 310, 452–455
Kupffer's cells. *See* Stellate cells

Labial glands, 115, 123
Labyrinth, membranous, 934–945; cells of Boettcher, 943; cells of Claudius, 943; cells of Deiters, 944; cells of Hensen, 944; crista spiralis, 936; ductus cochlearis, 934; ligamentum spiralae, 935; membrana basilaris, 936; membrane of Reissner, 938; osseous capsule of, 929–932; stria vascularis, 940
Lachrymal gland, 17, 36
Lactation, 1191; initiated by removal of ovaries, 1195
Lactation, increased demand for thyroxin, 568
Lactic acid and muscular contraction, 781, 788, 789, 798, 799, 836; in carcinoma, 1202
Lacunae of bone, 718
Lamellae of bone, 715–718
Lamina reticularis, 945–953
Lamina spiralis ossea, 932
Langendorff cells, 558

Langerhans: cells of epidermis, 32; enumeration of islands of, 255; islands of, 246–251; relation of islands to acini, 254–257
Lecithin in suprarenals, 647
Lens of epiphyseal complex, 511
Lens of eye: immune body production by, 17; literature on, 17
Lentiform body: of cones, 896; function of, 907; of visual rods, 893
Leptochromatic nuclei, 375
Leptomeninges, 1089–1095; mesothelial cells of, 1093; term defined, 1082
Leptotene stage of chromosomes, 1278
Leptotene threads, 1274
Lesser sublingual glands, 115
Leucemia, cultivation of mongranular leucocytes in, 351
Leucemia, lymphatic, 340; myeloblasts in, 381–385
Leucemia, myelogenous: basophiles in, 417; eosinophiles in, 414; megalocaryocytes in, 422; platelets in, 419; segmentation of megalocaryocytes in, 420
Leucoblasts, undifferentiated, relation to myeloblasts, 372
Leucocytes: basophile, 415–417; bibliography, 423–424; eosinophile, 411–415; granular, 403–424; megalocaryocyte, 421–422; neutrophile, 403–411; platelets 417–421
Leucocytes, large mononuclear, relation to monocytes, 458
Leucocytes, transitional, relation to monocytes, 458
Leucocytes in vaginal fluid, 1157ff.
Leucocytoid lymphocytes, 322
Leucomalanin, 30
Leucoplastids, 30
Leydig, cells of, 1223, 1232
Lieberkuehn's crypts, 171, 188
Ligamentum spiralae, 935
Ligation of pancreatic ducts, 261, 262, 265
Light: and periodicity in vital phenomena, 13; influence of, on skin, 17; protection of skin against, 15. *See* Ultraviolet
Lingual glands, 123
Lipase in intestinal cells, 177
Lipochrin in retinal epithelium, 903
Lipochrome: in the skin, 28; in suprarenal, 641–642; test for, 642
Lipofuscin, cortical pigment of suprarenal, 642
Lipofuscin in: retinal epithelium, 901; suprarenal cortex, 642
Lipoid in: prostate, 1311; suprarenal cortex, 646
Lipoid inclusions of: lutein cells, 1134; suprarenal cortex, 640, 646; testicular interstitial cells, 1219
Lipoidemia of diabetes, histiocytes in, 429
Lipoidhistiocytosis, 429
Lipoids, reactions of, 647–648
Lipolytic enzymes of lymphocytes, 353
Littoral cells of Siegmund, 449, 451, 455, 469
Liver, 205–233; cell specialization, 230; comparative anatomy, 206; cytology, 211–224; detoxicating action, 228; embryology, 206; hepatic extracts, 232; mammalian hepatic lobule, 207–211; pathology, 231; regeneration, 231; structural and physiological changes, 224–230
Liver cytology, 211–224; biliary channels, 218; cell structure, 211–213; changes during physiological activity, 215; mitochondria, 214; stellate cells, 217
Liver: in bilirubin formation, 309, 310; histiocytes of, 452–455
Locomotion of cell types, 8
Lung, histiocytes of, 455
Luschka's ducts, 222
Lutein cell: definition, 1132; degeneration, 1138; Golgi apparatus, 1132-1134; lipoids and neutral fat, 1134–1137; mitochondria, 1134; pigment, 1137; secretory granules, 1138
Lutein cells of testis, 1214
Lymphadenosis, acute, 322
Lymphatic leucemia, myeloblasts in, 383
Lymphatics: absent in meninges, 1101; of hypophysis, 490; of liver, 210; nasal, 57, 59; of pineal, 532; relation to sympathetic cells, 1019; salivary, 123; of synovial membrane, 747, 750
Lymphatics, rôle in absorption of cerebrospinal fluid, 1099–1100
Lymphoblast, 335, 348
Lymphocytes, 321–353; bibliography, 359–367; development, 328–330, 434, 439, 461; and defense reaction, 353; function, 353; germ centers, 330–341; interrelations, 341–342; origin in embryo, 344–345; potencies, 345–353; reticulum and histiocytes, 343–344; structure, 322–328; large, 322, 331, 332, 334; distinguished from macrophages, 445; relation to myeloblasts, 372; small, 322, 338; pseudopodia, 349; cytocentrum, 349; vacuoles in, 349
Lymphocytes in: nasal mucous membrane, 56; respiratory epithelium, 73; rôle in digestion, 177
Lymphocytoid polyblasts, 349
Lymphocytoid wandering cells, 345, 465
Lymphogonia, 331, 341
Lymphoid cells, indifferent relation to myeloblasts, 372
Lymphoid mother-cell, relation to myeloblasts, 344, 345
Lymphoid tissue, histiocytes of, 442–450
Lymphoid tissue, nasal, 57; germ centers of, 330–341
Lymphoidocytes, 358; relation to myeloblasts, 372, 385, 386

M line in: cardiac muscle, 824; striated muscle, 772

Maceration, methods of, 680
Macroblast, 276
Macrocyte, 276
Macrogenitosomia praecox, 541–542
Macroglia. *See* Astrocytes
Macrophages in follicular atresia, 1131
Macrophages or histocytes, 427–484; bibliography, 476–484; of common connective tissue, 429–436; development, 463–470; free in blood, 455–458; function, 474–475; genetic relations, 470–474; of leptomeninges, 1090–1094; of liver, 452–455; of lower vertebrates, 474; of lymphoid tissue, 442–450; of myeloid tissue, 450–451; of omentum, 436–442; regional differences, 455; relation to monocytes, 458–463; of spleen, 451; of synovial membrane, 740; terminology, 427–429
Macrophages, origin from reticular elements, 334
Macrophages and reticular histiocytes, 329
Macula, 953
Malaria, machropages in, 457
Malpighian corpuscle. *See* renal glomerulus
Malpighian layer. *See* stratum germinatium, 19
Mammary gland, epithelium phagocytic, 1202
Mammary glands, 1175–1208; activity cyclical, 1175; bibliography, 1204–1208; carcinomatous change in, 1202–1204; changes during estrous cycle, 1185–1187; classification of, 36; effect of removal of fetuses from uterus, 1188; experimental and pathological conditions, 1201–1204; growth during pregnancy, 1187–1188; influenced by myometrial gland, 1188; influenced by pineal extracts, 538; life cycle, 1180–1185; mechanism of secretion, 1192–1195; proliferation of, 3; retrogression, 1195–1198; secretion, 1181, 1188–1192; sexual cycle, 1176–1178; stroma changes, 1198–1201; structure, 1178–1180
Mammary glands in males, 1184; inhibiting action of testicles, 1184
Mankowski's granules, 249
Mast cells in mammary gland involution, 1200
Mast leucocyte. *See* Basophile leucocyte
Mastitis, chronic cystic, 1204
Masturbation, effect on intranasal erectile tissue, 58
Maturation of male germ cell, 1270–1292
Maturation of ovum, 1123–1125; relation to ovulation, 1124
Maxillary sinus, 60, 64
Mechanical irritation of gastric glands, 145
Mechanical protection by skin, 13
Meckel's diverticulum, 141
Medium, influence on structure of cells, 3
Medulloblasts of Bailey, 1047
Megaloblast, 276
Megalocaryocytes, 420, 421–422
Megacaryocytes in cultures, 348; relation to myeloblasts, 371
Megacaryocytes, platelets formed from, 300
Megalocyte, 276
Meibomian glands, 36
Melanin, 27–32; chemistry, 28; formation of, 29; in cutaneous glands, 36; inheritance of tendency to formation, 29
Melanoblasts, 32
Melanogens, 29
Melanoprotein, 28
Membrana basilaris, 936-938
Membrana granulosa: bibliography, 1147–1150; bodies of Call and Exner, 1129; in atresia, 1130; normal granulosa cell, 1128
Membrana tectoria, 949–953
Membrana vestibularis. *See* Reissner's membrane
Membrane bones: development of, 731; ossification of, 731
Membrane theory of nerve conduction, 978
Membranes. *See* Serous
Memory and nerve cells, reference to, 984
Mendelian dominants in blood groups, 295
Meningeal spaces: development, 1083–1085; terminology, 1082
Meninges, 1080–1083; absence of lymphatics in, 1101; terminology, 1081–1082
Meningitis, experimental, 1090
Menopause, thyroid enlargement in, 565
Menstruation caused by ovarian hormone, 1169
Menstruation, effect on: intranasal erectile tissue, 58; mammary stroma, 1200; related changes in mammary glands, 1186
Merocrine glands, 36
Mesoblast, 276
Mesoglia, 1055; abnormal forms, 1057
Mesonephros, 665–670; corpuscle, 668; tubule secretion, 669
Mesophragma of Heidenhain, 824
Mesothelial cells of leptomeninges, 1090–1094; relation to macrophages, 1090
Metabolic gradient, 979, 994
Metabolic rate increased in acromegaly, 565; in nerve cells, 980, 994
Metabolism of carbohydrates: in esosinophiles, 414; of fats in liver, 227; in histiocytes, 475; of iron in liver, 230; liver, 226; in lymphocytes, 353; of pancreas, 263–268; of proteins in liver, 227
Metabolism, total after thyroidectomy, 560
Metafibrils, 818
Metanephros: avian, 679; mammalian, 680–687; reptilian, 676–679
Metastases of calcium, 847
Metrocytes, 276
Microblast, 276
Microcyte, 276; in hemolytic jaundice, 282

Microdissection, introduction of technique, 6
Microglia, 1055; origin, 1058–1061; immigration, 1061; morphology and types, 1056; relation to reticulo-endothelial system, 1063
Microincineration, 7
Micromyeloblasts, 321
Micromyelocytes, 347
Microphages of Metschnikoff, 427
Milk secretion, 1188–1192
Millon's reagent applied to salivary glands, 100
Mingazzini phenomenon, 179
Mitochondria: basophiles, 416; cartilage, 705–707; cerebrospinal nerve cells, 971–974; demilunes of salivary glands, 104–105; derivation of term, 24; eosinophiles, 412; epidermis, 24; gastric glands, 141, 143–145, 152; and Golgi apparatus, 37; granulosa cell, 1128; histiocytes (chondriosomes), 431; intestinal cells, 176; lutein cells, 1134; lymphocytes (chondriosomes), 324; male germ cells, 1284ff.; mammary glands, 1192; megalocaryocytes, 422; monocytes, 459; myeloblasts, 378; neutrophiles, 407; of liver cells, 214; ova, 1119; pancreas, 244, 249; parathyroids, 580; platelets, 418, prostate, 1312; Purkinje's cells, 864; relation to keratohyalin, 32; relation to nutrition, 180; relation to protein metabolism, 227; relation to yolk formation, 1122; renal tubules, 689, 690, 691; salivary mucous cells, 101; seminal vesicles, 1307; skin disease, 25; striated muscle synovial membrane, 744; suprarenals, 623–624, 643–645; testicular interstitial cells, 1215, 1219; thymus, 593; thyroid, 559
Mixed leucemia, 384
Moderator band of heart, 876
Modiolus, 932
Molar glands, 123
Molecules, orientation of, 8
Moll's glands, 36
Molluscum contagiosum, inclusions in, 35
Mongolian idiocy, 569
Monocytes: definition of, 321; embryonic development, 463–470; genetic relations, 470–474; relation to histiocytes, 458–463. *See* Macrophages and histiocytes
Monomolecular layer, significance of, 8, 966
Monophyletic theory of blood formation, 297
Mucicarmine, stain, 184
Mucin in: cartilage matrix, 706; epidermis, 35; salivary glands, 94–98
Muco-perichondrium, 54
Muco-periosteum, 54
Mucous cells: of extrahepatic biliary ducts, 220; intestinal, 181; nasal, 55; salivary, 94–98
Mucous glands: identification of, 94–98; paranasal, 63; salivary, 93; secretion antecedent, 95
Mucous membrane. *See* Nasal mucous membrane and paranasal mucous membrane
Mucus, formation of, 183
Mulattoes, pigmentation of, 28
Muscle. *See* Smooth, striated and cardiac muscle
Mushroom poisoning, effect on erythrocytes, 310
Myelinated and unmyelinated fibers, 1016
Myelination, mesoglia in, 1062
Myeloblast, 297, 321; and lymphoblasts, 348
Myeloblasts, 371–400; Auer bodies, 380; azure granules, 379; bibliography, 391–396; biological characters, 380–381; conclusions, 390–391; definition, 371; in leucemias, 381–385; mitochondria, 378; in normal adults, 385–388; position in hematological system, 388–390; proteolytic ferment, 380; structure, 372–380; synonyms, 371
Myelogeneous leucemia, 382
Myeloid granules in retinal epithelium, 903
Myeloid tissue, histiocytes of, 450–451
Myeloidin in retinal epithelium, 903
Myenteric plexus, 1020, 1023
Myocarditis, syphilitic, Purkinje's cells in, 872
Myoepithelium of mammary gland, 1180
Myofibrils: origin of, 815–819; relation to contraction, 820, 821; size, 821, 822
Myoid: function of, 907, 908; of visual cones, 896; of visual rods, 893
Myoid cells of thymus, 595
Myometrial gland, effect on mammary gland, 1188
Myxedema: general effects, 566, 569; effect on erythrocytes, 282; reference to nerve cell changes, 971

N discs of cardiac muscle, 826
Nails, 37
Nasal fossa: olfactory portion, 48; respiratory portion, 53
Nasal mucous membrane, 47–60; basal cells, 52; basement membrane, 56; chemical composition, 65; corpora cavernosa, 58; glands, 59; olfactory cells, 49; olfactory portion, 48; protective mechanism, 64; respiratory portion, 53–60; sustentacular cells, 51; trigemmal nerve, 52; tunica propria, 52, 56
Nasal vestibule, 47
Nebenkern, 246, 1282
Neck chief cells of gastric glands, 155–157
Necrosis, focal, pancreas, 259
Negri bodies, 977
Nephritis, chronic, calcium metastases in, 847
Nephrostome, 664

Nerve cells, cerebrospinal, 965–987; bibliography, 984–987; cell membrane, 966; chromidial substance, 968–971; functional activity, 981–984; dynamic polarization, 978–981; Golgi apparatus, 974; mitochondria, 975; neurofibrils, 971–974; other normal components, 976; protoplasm of living cells, 967; reaction products, 977
Nerve cells, sympathetic, 1009–1029; axon, 1016–1018; bibliography, 1026–1029; cell capsules, 1019; changes in chromidial substance, 1024; chromidial substance, 1011–1014; classification, 1018, 1019–1023; dendrites, 1018; inflammatory and degenerative processes, 1025; morphology 1009; neurofibrils, 1010; nucleus, 1014; nucleus plasma ratio, 1015, pigmentation, 1023
Nerve supply of: cartilage, 709; epidermis, 34; hypophysis, 490; internal ear, 957; kidney, 700; olfactory epithelium, 52; parathyroids, 578; prostate, 1313; Purkinje system, 858; salivary glands, 115–123; smooth muscle, 795; striated muscle, 783; suprarenal, 629; synovial membrane, 750; testis, 1241; thymus, 593; thyroid, 557
Nerves: carbon dioxide production, 980, 982; electrical phenomena, 978, 980; oxidation rate, 980; secretory, 557; temperature coefficient, 981, 982; regeneration, 979
Nervous impulse, 981–984; coördination, 993, 1001–1004; correlation, 993, 1001–1004; course of, 993; diffusion, 999, 1001; integration, 999, 1000, 1004
Neurilemma, 1016
Neurocrine gland, 187
Neuroepithelium of vestibule and semicircular canals, 953ff.
Neuroepithelium of olfactory region, 48
Neurofibrils: continuity of, 1017; cerebrospinal nerve cells, 971–974; pathological changes in 974, 1026; sympathetic nerve cells, 1010
Neurofibrils in rods and cones, 898
Neuroglia: contacts, 1043–1044; development, 1044–1052; growth and degeneration, 1052
Neuroglia and microglia, 1033–1068; abnormal mesoglia, 1057; bibliography, 1065–1068; definition, 1033–1034; development of neuroglia, 1044–1052; function of interstitial cells, 1064–1065; microglia (or mesoglia), 1055–1057; neuroglia morphology, 1034–1044; origin microglia, 1058–1064; overgrowth and degeneration of neuroglia, 1052–1055
Neuroglia of pineal, 516, 536
Neuron theory, 966
Neuronophagia in infective disease, 1025
Neurons (see also nerve cells) relation, histiological character to function, 991–1005; association with astrocytes, 1044; bibliography, 1005; classification, 992; conclusion, 1004; correlation and coördination, 1001–1004; parts of neuron and relation to conduction, 993–995; perphieral neurons, 998–1001; relation of cell body and dendrites to central fatigue, 995–998
Neusser's perinuclear granules, 408
Neutral fat in: histiocytes, 432, 452; liver, 214; lutein cells, 1134; suprarenals, 647. *See also* Fatty substances
Neutral gentian stain, 184
Neutral red granules of histiocytes, 438
Neutrophile leucocytes, 403–411; cytoplasm, 406–408; fate, 411; function, 410; motility, 408–409; nucleus, 404–406; number, 403; origin, 409–410; size, 404
Nicotine, effect on testicles, 1240
Nissl bodies. *See* Chromidial substance
Nitroprusside reaction, 37
Nonius field, 838
Non-nucleated plates Kölliker, 78, 82
Non-polar substances, 279–280
Normoblast, 276
Normocyte, 276
Nucleic acid, abundant in thymus, 600
Nucleoids, 307
Nucleolar changes in epidermis, 32
Nucleus: maturation in eggs, 1123–1125; in sympathetic nerve cells, 1014
Nucleus of: epidermal cells, 24; pancreatic cell, 246; testicular interstitial cell, 1215
Nucleus-plasma ratio: Hertwig's law of, 1015; sympathetic nerve cells, 1015
Nuel, space of, 945, 947, 949
Nüssbaum cells, 161
Nutrition, effect on bones, 719

Oddi's sphincter, 221
Oehl's layer. *See* Stratum lucidum, 20
Oil globules in color perception, 911
Olfactory cells, 49–51
Olfactory glands, 52
Oligodendroglia: derivation of term, 1041; interfascicular glia, 1042; perineuronal satellites, 1042; satellitosis, 1055
Oligoglia: cytogenesis, 1048; function, 1065
Omentum, histiocytes of, 436–442
Ontogenesis, 713; erythrocytes in, 305
Osmotic properties of: smooth muscle, 795–798; striated muscles, 784–787
Ossification, 728, 731
Osteoblasts, 713, 724, 726, 730
Osteoclasts, 713, 724
Osteomalacia, effect on parathyroids, 584
Osteoporosis, effect on parathyroids, 584
Otic ganglion, 1020
Otolith membrane, 938, 957
Otosclerosis, 930
Ovarian hormone, effect in menstruation, 1169
Ovary: changes corresponding to character of vaginal fluid, 1167–1169; relation to

suprarenals, 650. *See* Lutein cells. *See* Interstitial cells of
Ovary, extirpation of, effect on mammary glands, 1181
Ovulation: conditioned by copulation, 1176; spontaneous, 1176
Ovum, 1111–1128; bibliography, 1145–1147; crystalloids, 1120; degeneration of unfertilized ovum in tube and uterus, 1127; degeneration in follicle, 1125–1127; formation, zona pellucida, 1115; Golgi apparatus, 1117–1118, 1122; homologies, zona pellucida, 1116; mitochondria, 1119; nucleus, maturation, 1123–1125; size, 1111–1112; structure, zona pellucida, 1113–1114; yolk, 1121; yolk formation, 1121–1123
Oxidations: and muscular activity, 769; and nervous activity, 980
Oxidizing enzymes of leucocytes, 353; of myeloblasts, 381
Oxydase reaction: eosinophile leucocytes, 412; nerve cells, 979; neutrophilic leucocytes, 408
Oxygen consumption, blood, 292
Oxygen supply, influence on: cell division, 1193; growth and retrogression, 1196; keratinization, 33
Oxygen tension in blood, 291
Oxyhemoglobin, 291
Oxyphil cells of parathyroids, 579

Pacchionian bodies, 1097
Pachychromatic nuclei, 375
Pachytene stage of chromosomes, 1278
Pacinian corpuscles, 16; in synovial membrane, 751
Pain sensations, 16
Palatine glands, 123
Pancreas, 241–271; acinous cell, 241–246; centroacinous cells and duct cells, 251–253; chromidial substance, 243; fat globules, 245, 250; Golgi apparatus, 244, 250; histogenesis, 253; islands and carbohydrate metabolism, 263–268; islet cell, 246–251; ligation of ducts, 261; mitochondria, 244, 249; nucleus, 246; reaction to injury, 257; regeneration, 260; relation of acini to islets, 254–256; specific granules, 246–249; zymogen, 241–243
Pancreatectomy, 265
Pancreatic duct ligation, 261, 262
Pancreatic juice, 243
Paneth cells, 183–185, 161
Papilla spiralis, 943, 945–953
Parachromatin, 374
Paraeleidin, 34
Paraganglia, chromaffin cells in, 625
Parakeratose. *See* Keratohyalin
Paralytic secretion, salivary glands, 104
Paranasal sinuses, mucous membrane, 60–64
Paranucleas in pancreatic cells, 246
Parapineal organ, 504
Paraplasm, 356
Parasmypathetic system, 1009, 1015
Parasympathetic terminals in salivary glands, 115–123
Parasynapsis, 1279
Parathyroid glands, 575–588; bibliography, 585–588; blood chemistry of tetany, 582; blood supply, 578; and calcification, 719; classification, 578; comparative anatomy, 576; embryology, 575; general morphology, 577; innervation, 578; interrelations, 585; physiology, 581; regeneration and hypertrophy, 583; secretory epithelium, 579–581; terminology, 575; transplantation, 584
Parathyroid tetany, symptom complex, 581
Parietal cells, 140, 157–160, 162
Parotid gland of man, 125, *See* Salivary glands
Pars distalis of hypophysis, 491–493
Pars intermedia of hypophysis, 493–494
Pars tuberalis of hypophysis, 494–495
Pathology of: cardiac muscle, 845–847; cerebrospinal nerve cells, 974ff.; liver, 231; mammary glands, 1201–1202; Purkinje cells, 881; 883, striated muscle, 783; sympathetic nerve cells, 1023–1026
Pepsin in gastric mucous membrane, 148
Peptic cells, 140
Peptone shock, effect on erythrocytes, 282
Perichondrium, 705
Pericytes of liver capillaries, 452
Perilymphatic spaces, 932–934
Perimysium, 884
Perineuronal satellites. *See* Oligodendroglia
Perineuronal spaces, 1086–1089
Periodicity in vital phenomena, 13
Periosteum, 705
Peritoneal cavity, infections of, 440
Perivascular spaces, 1086–1089
Permeability, problems in, 7; of erythrocytes, 291, of skin, 15
Pernicious anemia, effect on: decrease of grant cells in, 420; decrease of platelets in, 418, 419; erythrocytes, 282, 304, 305; lobation of leucocytes in, 411
Peroxydase reaction: eosinophile leucocytes, 412; neutrophilic leucocytes, 408
Perspiration in reduction of temperature, 16
Peyer's patches, 171
Phagocytes, mononuclear and bone resorption, 725
Phagocytosis by: acinous cells, 258; eosinophilic leucocytes, 414; intestinal epithelial cells, 180; macrophages, 447; mammary gland epithelium, 1202; neutrophilic leucocytes, 410; osteoclasts, 724; stellate cells, 414; thymic cells, 594
Pharyngeal tonsils, 57
Phenolase of skin, 17
Phenoltetrachlorphthalein, excretion of, by liver, 565
Phenylhydrazine, effect on lymphocytes, 339

Phosphatase of skin, 17
Phospholipin of erythrocytes, 290
Phosphorus of blood increased during tetany, 583
Phosphorus poisoning, pancreas, 258
Photochemical theory of vision, 905–907
Photoreceptors, 905
Photosensitive substances, 904–906
Physical characters, erythrocytes, 275–289
Physico-chemical changes in contraction, 835–837
Physiological degeneration of pancreas, 268
Physiological inflammation: during estrous and ovulation, 1169; omentum, 440
Pia arachnoid, term defined, 1082
Pia: relation to astrocytes, 1043; term defined, 1082
Pigment in: astrocytes, 1037; biliary system, 225; cardiac muscle, 830, 841; hypophysis, 496; histiocytes, 343; liver, 207, 225; lutein cells, 1137; nerve cells, 976; paranasal sinuses, 63; pineal, 518; 525; prostate, 1312; Purkinje cells, 875; retina, 899–914; seminal vesicles, 1307; suprarenals, 640, 652; sympathetic cells, 1023; testicular interstitial cells, 1220; visual rods, 894
Pigment; flow of, 900, 901, 908; origin of, in retina, 903
Pigmentation: in Addison's disease, 640; carotinoid, 28; influenced by pineal feeding, 537; influence of suprarenals on, 652; melanic, 27–32; rôle of enzymes in, 29; stimulated by light, 15
Pillar cells of internal ear, 945, 948
Pilocarpine, effect of, 176; on pancreatic secretion, 244, 246, 256; on salivary demilune cells, 106
Pineal extracts, 538
Pineal gland, 503–548; bibliography, 543–548; clinico-pathological evidence, 541–542; comparative anatomy, 504–505; comparative cytology, 506–537; comparative embryology, 505, 506; conclusions, 542–543; physiological significance, 537–541
Pineal therapy, 538
Pinealectomy, 539–541
Pituitary. *See* Hypophysis
Pituitary membrane, 48
Placenta, production of, 1176
Placentomata, 1185, 1186, 1188
Plasma cells, 353–367; atypical, 358; bibliography, 359–367; motility, 355; size, 354
Plasmamast cells, 359
Plasmophore of Holmgren, 827
Plasmosomes, 246
Plastochondria. *See* Mitochondria
Plastosomes. *See* Mitochondria
Platelets, 471–421; distribution and number, 418–420; function, 421; origin, 420
Platelets and lymphocytes, 327
Platelets, origin from megacaryocytes, 300
Plexus cavernosi concharum, 58
Plicae circulares, 171
Pneumonia, megalocaryocytes in, 422
Pneumonic exudate, 81, 84
Poikilocyte, 276
Poisoning: mushroom, 311; phosphorus, 258
Poisons, effect on liver, 232
Polar bodies, 1123–1125
Polar plasm, defined, 1261
Polar spindles, 1123, 1124
Polarity of epidermal cells, 31
Polarization, dynamic, of nerve cells, 966, 978–981
Polarized light: appearance of muscle in, 782; epidermis in, 21; examination of visual rods in, 891; nails in, 37
Poliomyelitis, chromidial substance in, 976, 1024; mitochondria in, 976, 1024
Polyblasts developed from resting wandering cells, 435
Polyblasts relation to monocytes and lymphocytes, 349; relation to fibroblasts, 358
Polychromatophilia, 308
Polycythemia: basophiles in, 417; effect on hemoglobin, 287; eosinophiles in, 414
Polyphyletic theories of blood formation, 297
Pores, alveolar, 85
Postbrancial bodies, 553, 588
Postmenstrual period, mammary gland during, 1186
Potassium in epidermis, 35; in nerve cells, 979, 980
Potassium salts, relation to muscular irritability, 796
Preameboid cells. *See* Oligodendroglia
Precipitin test for thyreoglobulin, 560
Precipitins, 290
Pregnancy, effect on: erythrocytes, 288; growth of mammary gland during, 1187–1188; hypophysis, 497; lipoid inclusions in lutein cells, 1134; repeated, leading to carcinoma of breast, 1203; sedimentation rate, 289; suprarenals, 649–652; thyroid regeneration, 563, 565
Premenstrual period, mammary gland during, 1186
Pressure sensations, 16
Prezymogen of Mouret, 141
Primordial germ cells, 1262, 1263, 1266
Principal cells of intestine, 172
Prochromosome stage of chromosomes, 1278
Procrythroblast, 276
Prohematoblast, 276
Prolymphocytes, 340
Promegaloglast, 297
Prominentia spiralis, 939
Promyelocyte, 379
Pronephros, 664
Pronormoblast, 276, 297
Proper gastric glands, 139
Proprioceptive reactions, 1003
Proprioceptive sensation, 992
Proprioceptors of Sherrington, 16

Prostate, 1308–1313; epithelium, 1309; lipoid, 1311; secretion granules, 1310; size, 1309
Prostate concretions, 1309, 1311, 1312
Protection by skin against: fluids and gases, 15; light, 15; mechanical injury, 13; pathogenic organisms, 14; temperature, 16
Protective mechanism, nasal mucous membrane, 64
Protein absorption, 178–180; mitochondria in, 179
Protein metabolism, 227
Protein sensitivity, 18
Proteolytic enzymes: of leucocytes, 353, 410; of myeloblasts, 380
Prothemoblast, 276
Prothrombin, relation to platelets, 421
Protomeres of Heidenhain, 818
Protoplasm, fragility, 3
Prozymogen of Macallum, 141, 150
Pseudo-hermaphrodites, male: mammary gland in, 1184; testicular interstitial cells in, 1231
Puberty: appearance of crystalloids at, 1222; thyroid enlargement in, 565; thymic involution at, 598
Pure culture of cells, 5
Purkinje system of heart, 853–886; distribution, 857–860; histological, 853–856; special features of cells, 860–862
cells: adult type, 871–879; bibliography, 884–886; cleavage of Purkinje cells, 879–881; embryonic type, 862–871; fibrillae, 865–870, 876, 883; pathological, 881, 883; summary, 882; transitional cell types, 881
Purkinje cells: fatty degeneration, 884; resembling cardiac cells, 873–874; transformed into cardiac cells, 882
Purpura hemorrhagica, platelets in, 410, 421
Pyloric glands, 139

Q discs in cardiac muscle, 824
Q granules, 827, 828

Rabies, reference to neurofibrils in, 974
Radiant energy, storage of, 905
Radium: effect on testicles, 1236; reference to effect on nerve cell, 971
Ranvier's filaments d'union. *See* Intercellular bridges
Reaction products, nerve cells, 977
Receptors, contact and distance, 16
Recurrent fever, macrophages in, 457
Refracting substance of cardiac muscle, 839
Regeneration: bone marrow, 297; cardiac muscle, 843–845; gastric glands, 162; of liver, 231; of lymphocytes, 328, 353; of nerve fibers, 979; of pancreas, 260, 262–263; parathyroids, 583; respiratory epithelium, 73; synovial membrane, 752; thyroid, 563; uterine epithelium, 1164, 1165
Reissner's membrane, 932, 936
Renal glomeruli: mesonephric, 668; metanephric, 683, 687; phonephric, 664
Renal tubules, 663–702; avian metanephros, 679; bibliography, 700–702; blood vessels, 697; collecting tubule, 693; cytology, mammalian renal tubule, 687–700; elimination by, 689; glomeruli, 668; innervation, 700; mammalian metanephros, 680–687; medullary loop, 692; mesonephros, 665–670; metanephros, 670–687; neck segment, 688; pronephros, 664; proximal convoluted segment, 688–691; reptilian metanephros, 676–679; secretion, 669; segments of, 682–687; types of epithelium, 694
Resonance, physical, 938
Resorption: of bone, 724–727; and osteogenesis, 730
Respiration by skin, 15
Respiratory epithelium, 71–86; alveolar, 78–85; basal membrane, 77; bronchiolar, 74; regeneration, 73; stratified squamous, 73; tracheal and bronchial, 71
Respiratory portion of nasal mucous membrane, 53–60
Resting stage of chromosomes, 1278
Resting wandering cells of Maximow, 427, 435. *See* Macrophages
Rete Malpighi. *See* Stratum germinativum, 19
Rete mucosum. *See* Stratum germinativum, 19
Retesolution of Golgi apparatus, 975
Retespersion of Golgi apparatus, 975
Reticular cells: large stellate and spindle shaped, 328; of lymphoid tissue, 442, 443; of thymus, 594
Reticular histiocytes and macrophages, 329
Reticular material. *See* Golgi apparatus
Reticular syncytium, 336
Reticulated cells of hypophysis, 493
Reticulin fibers, 328, 329; of spleen, 451
Reticulocytes, 304; percentage in various animals, 304, 306; relation to oxygen consumption, 292
Reticulo-endothelial cells of thymus, thyroid and parathyroids, 579; relation to mesoglia, 1063
Reticulo-endothelial system in blood destruction, 310; term introduced, 428
Reticulum, alveolar, 77, 85; of lymph nodes, 442, 450
Reticulum, relation of: lymphocytes to, 343, 386; myeloblasts to, 390
Retina of epiphyseal complex, 508, 511
Retinal cells. *See* Visual cells
Retrogression of mammary gland, 1195–1198
Retrolingual gland of dog, special serous cells, 111

Reversible hemolysis, 296
Rhagiocrine cells of Renaut, relation to histiocytes, 435
Rickets, enlargement of parathyroids in, 584; and thymectomy, 599
Rivinian ducts, 129
Roentgen ray, effect on testicles, 1236
Roentgen rays, effect on thymic cells, 321
Rollett's cells, 140
Rosette: of histiocytes, 438; of monocytes, 459
Round wandering cells, 431
Ruffini's corpuscles, 16

Saccule, 953
Saccus endolymphaticus, 931
Säftkanalchen. *See* Golgi apparatus
Saliva, variation in composition, 119
Salivary glands, 91–135; blood supply and lymphatics, 123; classification, 92–94; development, 115; duct elements, 113–115; innervation, 115–123; of man, 123–129; mucous cells, 94–98; serozymogenic cells, 98–102; special serous cells, 102–113
Saponin, effect on: erythrocytes, 294; testicles, 1240
Sapotoxin, effect on lymphocytes, 339
Sarcolemma: of cardiac muscle, 837–839; of striated muscle, 785
Sarcolysis, 816
Sarcomeres, 772ff.: of cardiac muscle, 827; size, 775
Sarcoplasm, 817, 839; of Purkinje cells, 875
Sarcosomes, 827
Sarcostyles, 772ff.: changes in length, 787, 797; changes in volume, 781
Satellitosis of oligodendroglia, 1055
Scar formation, macrophages and polyblasts in, 468
Schistocytes of Ehrlich, 276, 279, 310
Schmidt's optically homogeneous zone. *See* Stratum lucidum, 20
Schneiderian membrane. *See* Nasal mucous membrane
Schultze's "Rippel." *See* Intercellular bridges
Schwann cells, 1064
Sciences, relation to cytology, 3, 9
Sclerosis, relation to islands of Langerhans, 261
Seasonal variations, testicular interstitial cells, 1226–1229
Sebaceous glands, 36; in nasal vestibule, 48
Sebum, 15
Second contraction stage of chromosomes, 1278
Secretine effect on pancreas, 256, 257
Secretion of: connective tissue, 435; hypophysis, 497; pancreas, 242–243; parathyroids, 579–581; thyroid, 559
Sedimentation rate, erythrocytes, 288
Segmentation of cardiac muscle, 842; Purkinje cells, 879–881
Segregation apparatus, 324
Semicircular canals and vestibule, 953–959; cupola, 958; macula, 953; membranous semicircular canals, 957
Seminal vesicle secretion, effect on vaginal contents, 1161
Seminal vesicles, 1303–1308; epithelium, 1304; mitochondria, 1307; secretion granules, 1305–1307
Seminal vesicles and pinealectomy, 540
Semipermeability, a surface phenomenon, 8; of smooth muscle, 796, 797; of striated muscle, 785, 786, 797
Sensations, exteroceptive, interoceptive and proprioceptive, somatic, visceral, 992
Sense organs of skin, 16
Sera, specific, sensitization, 280
Serous cells, special of salivary glands, 102–113
Serous exudate, alveolar, 83
Serous exudate in lungs, 81
Serous glands, of biliary ducts, 220; paranasal, 63; salivary, 93
Serous membranes, histiocytes of, 436–442
Serozymogenic cells of salivary glands, 98–102
Sertoli cells in: ectopic testes, 1231; testicular grafts, 1234
Serum lipoids, influence on keratinization, 34
Sex differences, thymus, 592
Sex glands: during hibernation, 1229–1230; effect of removal on thyroid, 565
Sexual apparatus, relation to: hypophysis, 497; intranasal erectile tissue, 58
Sexual cycle, phases of, 1176–1178
Sexual maturity and the thymus, 598
Shapiro's ereidisms. *See* Intercellular bridges
Siderophile cells of suprarenals, 643
Sino-auricular node, 853ff.
Sinus wall, cells of, 447–448
Sinuses, paranasal (maxillary, frontal and sphenoidal), 60–64; basal membrane, 62; epithelium, 61; glands, 63; tunica propria, 62
Sinusoids of liver, 209; histiocytes of, 452–455
Size of: erythrocytes, 277, 280, 283; hepatic lobule, 208; ova, 1111–1112; parathyroids, 578; prostate, 1309; suprarenals, 627; sympathetic neurons, 1009; testicular interstitial cells, 1211; thymus, 589; thyroid, 555
Skin: allergic reaction, 18; as an adaptive mechanism, 13ff.; architecture of, 13ff.; bibliography, 37–43; coloration, 27; effect of emotions on, 18; effect of fluids and gases on, 15; effect of thyroidectomy on, 561; enzymes of, 17; immune bodies, 17; influenced by internal organs, 18; pigmentation of, 30; researches on, 19; respiration and excretion by, 15; whitening of, 29

Skin glands; 36; literature on, 37
Skin grafts, 15
Smooth muscle, 789–802; bibliography, 801–804; biochemistry, 789–799; changes during contraction, 792–795; conclusions, 799–801; osmotic properties, 795–798; portions not connected with contraction, 795; structure of vertebrate, 789–792
Smooth muscle of biliary tract, 221
Somatic nerve cells, defined, 992
Sound waves, reception of, 938
Specific inclusions of epidermis, 35
Spectrophotometric studies, bilirubin, 309, 310
Spermatid, development of, 1287; flagellum, 1288
Spermatozoa: maturation, 1027–1292; morphology, 1292–1293; parts of, 1293; phagocytized by leucocytes, 1160
Sphendoidal ostium, 63
Sphenoidal sinus, 60, 63
Sphenopalatine ganglion, 1020
Sphincter of Oddi, 221
Sphingomyelin of erythrocytes, 290
Spider cells. *See* Astrocytes
Spleen, histiocytes of, 451
Splenectomy, effect on erythrocytes, 288, 293, 309
Splenocytes, 309
Spongioblasts, embryonic, 1045ff.
Spongiocytes of suprarenals, 641, 645
Spongioplasm, 356
Squamous epithelial cell nests in hypophysis, 495
Staining of living cells, possibilities of, 7
Status lymphaticus, 591, 598, 602
Stellate cells of liver, 217, 452–455; in iron metabolism, 230
Stellate cells of skin. *See* Langerhans cells
Steno's gland, 60
Stenson's duct, 127
Sterility and testicular interstitial cells, 1242
Stimuli, effect: on bone, 723, 724; sympathetic cells, 1013
Stippling, basophilic, 307
Stohr cells, 161
Stomach. *See* Gastric glands
Stratum corneum, synonyms for, 21
Stratum disjunctum, *See* Stratum corneum, 21
Stratum germinativum, 19; synonyms of, 19
Stratum granulosum, 20; synonyms, 20
Stratum intermedium. *See* Stratum lucidum.
Stratum lucidum, 20; synonyms for, 20
Striated muscle, 769–789; bibliography, 802–804; changes during contraction, 777–781; comparison, 774–777; conclusion, 799–801; innervation, 1017, 1018; osmotic properties, 784–787; pathology, 783; portions not connected with contraction, 783; structure and biochemistry, 787–789; uncontracted insect's wing, 771–774; vertebrate muscle fiber, 769–771; views regarding contraction, 781–782
Striated muscle in pineal, 517
Striations, transverse of cardiac muscle, 822–827
Stroma of: erythrocyte, 279, 290; thymus, 597
Subarachnoid space, 1082; fluid of, 1084; relation to subdural, 1085, 1086
Subdural space, 1082; relation to subarachnoid, 1085
Sublingual gland, 109; of man 128–129
Submaxillary glands: cat and dog, 104–107; insectivors, 111–113; man, 113, 123–125, 127; rabbit, 117; rodents, 107–111
Sudoriferous glands. *See* Sweat glands
Sugar puncture of Claude Bernard, 630
Sulfatase of skin, 17
Sulfhydryl constituent of skin, 30
Superior proplasm of Prenant, 100
Suprarenal bodies, 623–659; bibliography, 653–659; changes due to infections, 639; cortical inclusions, 651–653; cortex, 640–653; epinephrin inclusions, 631–640; hypertrophy, 648–651; innervation, 629; lipoid inclusions, 646; medulla, 625–640; pigment, 640–642, 652; volume and weight, 627
Suprarenalectomy: causes thymus regeneration, 600–601; effect of subsequent thyroidectomy on, 564; influence on antibody formation, 599; influence on resistance to infection, 639; influence on thymus, 598
Surface area, erythrocytes, 287
Sustentacular cells of nasal mucous membrane, 51
Sweat glands, 36; functions of, 15, 16; in nasal vestibule, 48
Swelling of muscle, 785, 786
Sympathetic ganglia: atrophy of, 1024; pigmentation of, 1024
Sympathetic nerve cells. *See* Nerve cells sympathetic
Sympathetic plexuses, 1020, 1022
Sympathetic terminals in salivary glands, 115–123
Sympaticotropen gland, 187
Synapse: defined, 966; irreversibility of conduction at, 978, 995
Synapsis, process of, 1274
Synapsis stage of chromosomes, 1278
Syncytia formation, 1193, 1194
Synovial cells: 742–745; absorption from joints, 751; bibliography, 754–755; blood vessels, 749; chromidial substance, 745; cytoplasmic constituents, 744; fat, 744; fluid, 753; folds and villi, 746–748; Golgi apparatus, 745; lymphatics, 750; mitochondria, 744; nerve, 750; normal variations, 751; regeneration, 752; in silver preparations, 745;

stomata, 750; tendon sheaths, 754; transition to cartilage, 748–749
Synovial exudate, 752
Synovial fluid: cells in, 753; Wassermann reaction, 752
Synovial membrane of joints and bursae 737–755; adipose types, 741; areolar type, 739; distribution, 738; fibrous type, 740; structure, 738
Syphilitic osteochondritis, cartilage growth in, 708

Tachycardia in exophthalmic goiter, 569
Telophragma of Heidenhain, 824
Telosynapsis, 1279
Temperature changes: effect on chromophile reaction, 632, 636; effect on ectopic testicles, 1235, 1295; effect on erythrocytes, 278; effect on muscle, 777–778, 798; effect on position of retinal elements, 912; protection by skin against, 16
Temperature coefficient of nerve impulse, 981, 982
Temperature regulation: effect of injury of suprarenals on, 564; influenced by thyroidectomy, 561; rôle of suprarenals in, 636; the skin in, 16
Temperature sensations, 16
Tendon sheaths, 754
Terminology, erythrocytes, 276
Testicle, hormone of, 3
Testicles: degeneration in, 1294; effect of increased temperature on, 1235; interstitial cells of, 1230; non-descended, hormone of, 1184, 1231
Testicles influenced by: cross breeding, 1241; deficiency diets 1238, 1296; deinnervation, 1241; disease, 1295; drugs and gland extracts, 1239; inanition, 1237; over feeding, 1236; partial castration, 1233; radium, 1236; restriction of blood supply, 1241; Roentgen rays, 1235, 1296, 1297; temperature, 1235, 1295; vasectomy, 1232, 1294
Testicles, inhibiting action on mammary glands, 1184; Roentgen rays, 1235
Testicular grafts, 1234, 1296
Testicular wounds, healing of, 1236
Testis. *See* Interstitial cells of. *See* Germ cells, male
Tetany, blood chemistry of, 578; fetal, 575; various forms of, 581
Tetrad structure, 1274
Theca interna, 1138–1141
Thrombocytes. *See* Platelets
Thrombus formation and platelets, 421
Thymectomy, 599, 601
Thymic cells, small, 593
Thymus, 388–607; age involution 597; bibliography, 603–607; blood supply, 592; cystic spaces, 595; embryology, 588–591; eosinophile cells, 596; general morphology, 591; Hassall's corpuscles, 594; innervation, 593; interrelations, 600; lymphatics, 593; myloid cells, 595; pathology, 601–603; physiology, 598–600; reticular cells, 594, small thymic cells, 593; stroma, 597
Thyreoglobulin in lymph, 560
Thyroid adenomata, relation to endemic goiter, 559
Thyroid extract, effect on testicles, 1240
Thyroid feeding, effect on: suprarenals, 649; thymus, 601
Thyroid gland, 551–575; bibliography, 570–575; blood vessels, 556; comparative anatomy, 551–552; developmental defects, 554; effect of removal, 560–563; embryology, 552–554; exophthalmic goiter, 569; follicles, 557; interfollicular epithelium, 558; interrelations, 564–566; myxedema, 569; nerves, 557; normal anatomy, 555; pathology, 566–568; physiology, 560; regeneration, 563; secretion and excretion, 559; simple goiter, 568; transplantation, 563
Thyroid vesicles compared with cavities in hypophysis, 494
Thyroidectomy: after suprarenalectomy, 564; effect on erythrocytes, 288; effect on hypophysis, 565; general effect, 560–563; influence on suprarenals, 649
Thyroparathyroidectomy, effect on: suprarenals, 631, 649; thymus, 601
Thyroxin, 562ff.
Tigroid bodies. *See* Chromidial substance
Tissue culture: and bioelectric forces, 980; introduction of method, 5; lymphocytes in, 342; of mammary gland, 1201; of nerve cells, 966; of retina, 891; skin in, 33
Tonofibrils, 25
Tonofibrils of Marcus, 822
Tonsils, pharyngeal, 57; plasma cells in, 356
Tracheal epithelium, 71–74
Trachoma, inclusions in, 35
Trachychromatic nuclei, 323, 375
Transfusion of blood, 294
Transitional leucocyte, 403; and monocyte, 458
Transplantation of: parathyroids, 584; testis, 1296; thyroid, 563
Trigeminal nerve, 52
Trophospongium. *See* Golgi apparatus
Tropochrome cells, 93, 106, 108
Tryptophane, 624; and thyroxin, 563
Tubercle bacilli, ingestion of, leading to epithelioid cell formation, 351
Tuberculin reaction, allergic, 18
Tuberculosis: effect on erythrocytes, 289; fluid of joints, 754
Tumor, effect of in pancreas, 261
Tumor formation, 4; change in metabolism, 1202; hereditary factors, 1203; and oxygen supply, 1193

Tumors of: appendix and intestine, 187; breast, 584, 1202–1204; hypophysis, 498; parathyroids, 584; pineal, 541–542; testicular interstitial cells, 1232; thyroid (adenomata), 559
Tunica propria of olfactory region, 52; paranasal sinuses, 62
Türks irritation forms, 358, 377
Typhus fever, testicular interstitial cells in, 1232

Ultimo-branchial body, 553
Ultraviolet light, effect on: melanin formation, 29, 31; nerve cell (reference to), 971; optic lens, 17; parathyroids, 584
Ultraviolet light, relation to melanin formation, 29
Ultraviolet photography, of nerve cells, 967, 969
Ultraviolet rays, study of, 7; absorbed by melanin, 30
Undulating membrane, 8
Unitarian conception of myeloblasts, 382
Unna's basal corneous layer. *See* Stratum lucidum
Unravelling stage of chromosomes, 1278
Urea, formation, 227; secretion, 689
Uterus, cyclical changes in, 1162–1167
Uterus, extirpation of, soon after ovulation, 1177
Utricle, 953

Vaccinia lesions, 25
Vagina, fluid content of: 1153–1171; bibliography, 1170–1171; cellular movements accompanying fluid changes, 1155–1162; cyclical changes in uterus and vagina, 1162–1167; estrous process, 1169–1170; fluid, 1154; ovarian changes, 1167–1169
Vaginal closure membrane, 1155
Valvula coli, 171
Variations in synovial membrane, 751
Variola, reference to inclusions in, 35
Vasectomy: effect on interstitial cells, 1232, 1233; influence on testicle, 1295; increase in vigor, 1233
Vasodilation and pineal extracts, 538–539
Venereal excesses, effect on intranasal erectile tissue, 58
Ventricle IV, 1078–1080
Ventricles, cerebral, 1071–1078
Vermiform process, 171
Vestibule and semicircular canals, 953–959
Villus: arachnoidal, 1095–1099; intestinal, 172; synovial, 746–748
Virus III disease, nuclear inclusions in, 35
Viruses, filterable, 35
Visceral nerve cells, defined, 992
Visual cells and retinal pigment, 889–926; cones, 895–897; development, 899; rod and cone fibrillae, 897–899; rods, 891–895
adaptations to life habits, 913–914; bibliography, 914–919; color vision, 911; duplicity theory, 911–918; functional interpretation, 907; miscellaneous activities, 912; photochemical theory, 905–907; untenable theories, 904
pigmented epithelium, 899–904; cell inclusions, 901–903; component zones, 900; origin of pigment, 903; function of retinal elements, 904–914
Visual green, 894, 896
Visual purple, 894, 906
Vital staining, histiocytic: reticular cells, 343; renal epithelium, 669, 690
Vitamin A deficiency and skin glands, 37
Vitamin A, influence on keratinization, 34
Vitamin B deficiency: nerve cells, 975; testicles, 1238
Vitamin C deficiency, testicles, 1238
Vitamin deficiency and nerve cells, 971
Vitamins and calcification, 719
Vitamins, effect of, 177
Vitreous of epiphyseal complex, 511
Vomeronasal organ, 60

Wandering cell, primary, 297
Warts, nuclear inclusions in, 35
Wassermann reaction of joint fluids, 752
Water absorption, 178
Weight of: muscle, changes in, 796, 798; parathyroids, 577; thymus, 589, 591–592; thyroid, 555; suprarenals, 627, 631
Wharton's duct, 127
White fibrous cartilage, 711
Wound, healing, 4

X ray, reference to effect on neurofibrils, 974
Xanthin element, 28

Y chromosome, 1282
Yellow elastic cartilage, 711
Yolk in eggs, 1121–1123
Yolk nucleus, 1121

Z line in: cardiac muscle, 823; striated muscle, 772, 780
Zuckerkandl's bodies, 625
Zymogen granule: definition of term, 149; in pancreas, 241–243

Paul B. Hoeber, Inc., 76 Fifth Avenue, New York

GENERAL CYTOLOGY

A Textbook of Cellular Structure and Function for Students of Biology and Medicine

EDITED BY

E. V. COWDRY

THIS was the first single volume to state comprehensively what is known or conjectured about the principles that govern cell structure and function, and to give data concerning the cell that are fundamental to the sciences of botany, zoology, physiology and pathology. The contents are all original studies, not quotations from previously published works, and they carry a significance for the scientific world hard to overestimate.

THE AUTHORS ARE

EDMUND B. WILSON
ALBERT P. MATHEWS
MERKEL H. JACOBS
RALPH S. LILLIE
ROBERT CHAMBERS
EDMUND V. COWDRY
WARREN H. LEWIS
MARGARET R. LEWIS
FRANK R. LILLIE
EVERETT E. JUST
EDWIN G. CONKLIN
CHARLES F. McCLUNG
THOMAS H. MORGAN

$7.50 *net* · POSTPAID $7.75

PUBLISHED BY
THE UNIVERSITY OF CHICAGO PRESS
CHICAGO ILLINOIS

IN PRESS · READY SPRING 1928

[UNIFORM WITH SPECIAL CYTOLOGY]

HANDBOOK OF MICROSCOPICAL TECHNIQUE

For Workers in Microscopic Anatomy, Containing Methods for both Plant and Animal Tissues

BY

WILLIAM H. F. ADDISON
EZRA ALLEN
JOSEPH L. T. APPLETON
ROBERT CHAMBERS
WILLIAM V. CONE
HAROLD J. CONN
EDMUND V. COWDRY
ULRIC DAHLGREN
HAL DOWNEY
SOPHIA H. ECKERSON
N. CHANDLER FOOT
ROBERT T. HANCE
CHESTER H. HEUSER
RAPHAEL ISAACS
JOSEPHINE W. McNABB
FRANK B. MALLORY
FREDERIC PARKER
WILDER PENFIELD
FLORENCE R. SABIN
PAUL G. SHIPLEY
ETHEL M. SLIDER
WILLIAM R. TAYLOR
DAVID H. WENRICH

EDITED BY

C. E. McCLUNG, PH.D.

Professor of Zoology, University of Pennsylvania; Director of Zoological Laboratory, University of Pennsylvania

CONTENTS

CHAPTER

PART I—GENERAL METHODS

I. SECTION AND NON-SECTION METHODS OF PREPARING MICROSCOPICAL SLIDES . *McClung*

PART II—SPECIAL METHODS

II. METHODS FOR FRESH TISSUES
- Physical agents
  - Microdissection
  - Microinjection . . . . *Chambers*
- Chemical agents
  - Vital stains . . . . *Foot*
  - Supravital stains . . . *Sabin*

III. BACTERIOLOGICAL METHODS . . . . *Conn, Mallory, Parker*

IV. BOTANICAL METHODS . . . *Taylor, Eckerson*

V. CYTOLOGICAL METHODS . . *McClung, Cowdry, Allen, McNabb, Hance*

CHAPTER

VI. EMBRYOLOGICAL METHODS . *McClung, Allen*

VII. HISTOLOGICAL METHODS
- Blood
  - Erythrocytes . . . . *Isaacs*
  - Leucocytes . . . . . *Slider, Downey*
- Bone . . . . . *Shipley*
- Teeth . . . . . *Appleton*
- Intercellular connective—tissue substances . . . *Mallory, Parker*
- Muscle tissues . . . . *Dahlgren*
- Nerve tissues . . . . . *Addison, Penfield*

VIII. PROTOZOOLOGICAL METHODS *Wenrich*

IX. FIXATION AND FIXATIVES . *McClung, Allen*

X. STAINING AND STAINS . . *McClung, Conn.*

XI. MISCELLANEOUS . . . . *McClung, Heuser*

PUBLISHED BY · PAUL B. HOEBER, INC.

SEVENTY-SIX FIFTH AVENUE · NEW YORK

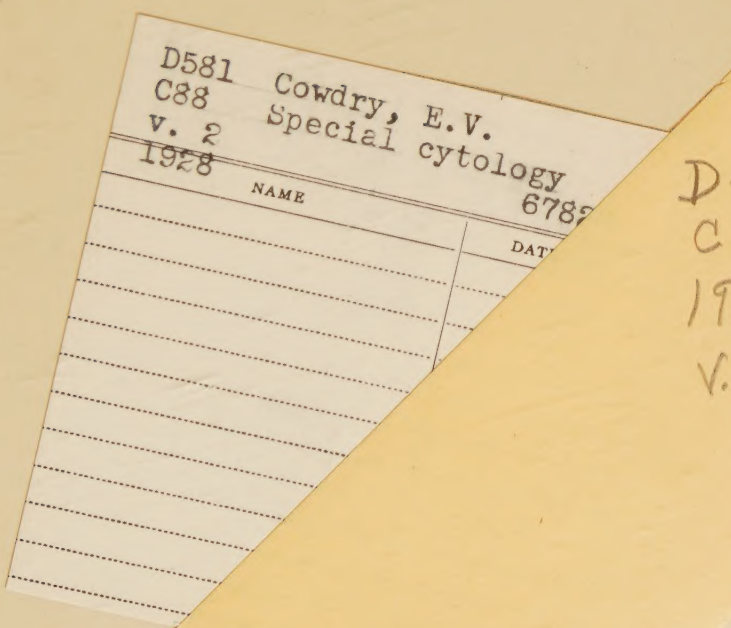
[PUBLISHERS OF SPECIAL CYTOLOGY]

LANE MEDICAL LIBRARY
300 PASTEUR DRIVE
PALO ALTO, CALIFORNIA 94304
Ignorance of Library's rules does not exempt violators from penalties.

JAN 16 1975
FEB 25 1992
FEB 17 1992

50M-10-63-5632

LANE MEDICAL LIBRARY
300 PASTEUR DRIVE
PALO ALTO, CALIF. 943



D581
C88
1928
v.2